AN ABSTRACT OF THE THESIS OF

Amelia C. Whitcomb for the degree of Master of Science in Fisheries Science presented on November 27, 2012.

Title: Mate Choice of Wild Spawning Coho Salmon (*Oncorhynchus kisutch*) in the Umpqua River, Oregon

Abstract approved: _____

Michael A. Banks Kathleen G. O'Malley

Evidence for reduced reproductive success (RS) of wild spawning hatchery-reared fish invites serious consideration with regard to the detrimental effects on subsequent generations of wild populations. Mate choice was evaluated as a potential mechanism contributing to these observed RS differences using a previous pedigree of wild spawning hatchery-reared and wild origin coho salmon (*Oncorhynchus kisutch*). Genetic variance at immune-relevant genes was used as a metric to examine mate choice. Two years (2005 and 2006) of three wild spawning mate pair classes were examined: wild x wild (W x W), hatchery x hatchery (H x H), and wild x hatchery (W x H). We tested for: (1) a departure from random expectations with regard to mate pair allelic diversity at immune-relevant markers, (2) a correlation between immune-relevant gene diversity and mate pair RS, and (3) distinguishable differences between mate

choice strategies used by hatchery-reared and wild origin coho. Eight immune-relevant gene-linked microsatellite markers were used to evaluate mate choice; four linked to immune-relevant expressed sequence tags (ESTs) and four linked to the Major Histocompatibility Complex (MHC). We found evidence for non-random mating between 2006 W x H mate pairs at *BHMS429*, an MHC-linked marker, and at *SsalR016TKU*, an immune-relevant EST-linked marker, which was identified as a vasodilator-stimulated phosphoprotein. Non-random mating was also evident between 2005 H x H pairs at *SsalR015TKU*, an immune-relevant EST-linked marker, though no putative gene was identified. All other pair classes did not display a significant mate choice signature. We found a significant correlation between mate pair RS and immune gene diversity among 2005 and 2006 W x W mate pairs as well as 2006 W x H mate pairs. Notably, H x H mate pair RS was not correlated to immune gene diversity in either year. Results suggest that mate choice and genetic compatibility may influence fitness of wild spawning coho.

Mate Choice of Wild Spawning Coho Salmon (*Oncorhynchus kisutch*) in the Umpqua River, Oregon

by

Amelia C. Whitcomb

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Amelia C. Whitcomb, Author

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Mate Choice of Wild Spawning Coho Salmon (*Oncorhynchus kisutch*) in the Umpqua River, Oregon

Chapter 1 – General Introduction

1.1 Hatchery and Wild Salmon Interactions

Hatchery and wild fish interactions have increasingly been studied to evaluate their role in the decline and limited recovery of wild salmon populations in the Pacific Northwest. Evidence that supplementing wild populations with hatchery-reared fish may be detrimental to the wild populations first became apparent in the 1980s (Waples, 1991). It has recently been shown that hatchery fish have lower reproductive success (RS) than wild fish when breeding in the wild (Araki et al., 2007, 2008) and that there is a carry-over effect from hatchery-reared fish to subsequent generations of wild populations (Araki et al., 2009; Christie et al., 2012a). The impact of hatchery-reared fish has been mostly documented in steelhead (*Oncorhynchus mykiss*) (Leider et al., 1990; Kostow et al., 2003; Araki et al., 2007, 2009), however this characteristic has recently been demonstrated in coho salmon (*Oncorhynchus kisutch*) as well (Thériault et al., 2011). Interestingly, the cause of fitness differences between hatchery and wild salmon remains unknown (Williamson et al., 2010).

Thériault et al. (2011) indicated that the point at which this difference in fitness between hatchery and wild coho salmon likely occurs is during mating. Their study compared fitness of hatchery coho released as unfed fry and smolts to wild coho

and found that both unfed fry and smolts had lower RS than wild coho. As the only distinction between the hatchery unfed fry and wild coho was spawning and incubation in the hatchery, they postulated that these disparities in fitness occur as a result of differences during mating (Thériault et al., 2011). Additional studies also suggest that fitness differences between hatchery and wild salmonids may be genetically based and result from an inability of hatchery crosses to replicate the complexity of mate choice as it occurs in the wild (Ford, 2002; Araki et al., 2007, 2008, 2009).

1.2 Relationship between Mate Choice and Reproductive Success

Mate choice involves recognizing a potential mate's genetic compatibility via various forms of sensory cues (Aeschlimann et al., 2003; Parrott et al., 2007). A mate preference for dissimilarity at genes that influence survival is thought to create a selective advantage for their progeny. For example, increased diversity at immune-relevant genes would enable offspring to initiate an immune response against a broader array of pathogens compared to individuals that are less diverse (Bernatchez and Landry, 2003). This advantage ultimately affects mate pair reproductive success (RS) and confers greater vigor to those offspring with genetic diversity at genes associated with survival.

The relationship between mate choice and RS has been examined in a variety of organisms. One study involving mice (*Mus musculus*) found that females accrued higher viability to their offspring when mating with a male they preferred,

based on behavioral discrimination, compared to a nonpreferred male (Drickamer et al., 2000). Spencer et al. (1998) found that female allied rock wallabies (*Petrogale assimilis*) chose mates for their genetic quality regardless of social pairing. In other words, in order to maximize RS, a female would choose to mate with another male in addition to the male she was socially paired with to increase the genetic quality of her offspring if the paired male was of poor genetic quality (Spencer et al., 1998). Male preference has also been evaluated in terms of RS. For instance owing to high reproductive cost of courtship and copulation in fruit flies (*Drosophila melanogaster*), a preference for a highly fecund (large) female has been observed among males and hypothesized to exist because current investment in mating reduces future mating opportunities (Byrne and Rice, 2006).

1.3 Major Histocompatibility Complex as a Metric for Mate Choice

Knowledge about associations between allelic diversity and fitness-related traits (such as disease resistance) has mostly resulted from studies of the Major Histocompatibility Complex (MHC) (Landry et al., 2001; Consuegra and De Leaniz, 2008; Evans et al., 2009, 2012). Olfactory cues have been shown to be involved in MHC discrimination, indicating a possible molecular mechanism that individuals may use to select mates based on body odor (Reusch et al., 2001; Olsén et al., 1998). The vertebrate MHC has been implicated in mate preference owing to potential genetic benefits that may be conferred to offspring (Neff et al., 2008; Milinski, 2006). The genes of the MHC encode proteins that are important in pathogen

recognition (Janeway et al., 2001). Therefore, the MHC plays an integral role in building an immune response and is under strong selective pressure (Klein, 1979; Potts et al., 1994).

Considerable research has focused on mate choice and MHC diversity in a wide range of species (for reviews see Jennions and Petrie 1997; Tregenza and Wedell 2000; Bernatchez and Landry 2003; Ziegler et al. 2005; Kempenaers 2007). In salmon specifically, many researchers have assessed the MHC genetic correlates of mate preference and RS (Landry et al., 2001; Pitcher and Neff, 2006; Forsberg et al., 2007). Landry et al. (2001) found evidence of mate selection based on increasing the heterozygosity of offspring at the MHC and Neff et al. (2008) found that Chinook (*Oncorhynchus tshawytscha*) females mated non-randomly with respect to MHC diversity. A previous study by Arkush et al. (2002) showed that Chinook salmon heterozygous at the MHC displayed higher pathogen resistance and similarly, Evans and Neff (2009) demonstrated that MHC class II heterozygous Chinook salmon fry exhibit fewer bacterial infections than do homozygotes. Lastly, Consuegra and De Leaniz (2008) established a causal link between mate choice in Atlantic salmon (*Salmo salar*), MHC diversity and increased parasite resistance to *Anisakis*, a marine nematode. All of these studies reinforce the idea that a heterozygous mating strategy is advantageous.

There have only been two previous MHC studies involving coho (Miller and Withler, 1997; Gómez et al., 2010). Miller and Withler (1997) found moderately high (0.7) MHC heterozygosity at the class IA locus. This was indicative of balancing selection and could be accounted for by nonsynonymous point muta-

tions. Gómez et al. (2010) compared the diversity and molecular evolution of MHC class II α and class II β and observed high levels of polymorphism with recombination and point mutation involved in generating diversity at positively selected sites. These studies provide additional evidence that a heterozygous mating strategy is advantageous.

1.4 Additional Immune-relevant Genes as Metrics for Mate Choice

Although the Major Histocompatibility Complex (MHC) is the most studied immune-relevant gene complex, examination of other immune-relevant genes is an appropriate approach to assess mate choice given that pathogen recognition and response is complex and likely involves gene products in addition to the MHC. These non-MHC immune-relevant genes may also experience different selection pressures and subsequently have different affects on offspring survival (Sommer et al., 2005). In spite of this, few studies have included non-MHC immune-relevant genes when evaluating the effect of mate choice on allelic diversity and reproductive success (RS) (Acevedo-Whitehouse and Cunningham, 2006). However, there has recently been a push in the field of immunogenetics to broaden the scope of research to include immune-relevant genes beyond the MHC in order to better understand how particular pathogens affect genetic diversity and how genetic diversity influences susceptibility or resistance to pathogens (Acevedo-Whitehouse and Cunningham, 2006). A mapping study in humans revealed that approximately half of the genetic variability for resistance to infection is attributable to non-MHC genes (Jepson

et al., 1997). The inclusion of immune-relevant genes in addition to the MHC when evaluating mate choice enables new perspectives on the functional relevance of alternate polymorphisms and may contribute to identification of other linkages or associations with fitness differences within and among populations of hatchery and wild salmon. Findings from such studies may ultimately inform and help improve strategies to increase fitness of hatchery fish and overall salmon recovery.

1.5 Research Goal and Objectives

The aim of this study was to determine (1) whether coho discriminate between immune-relevant genotypes when choosing their mates, (2) whether that choice is correlated to increased reproductive success (RS), and (3) if there is a distinguishable difference between mate choice strategies used by hatchery-reared and wild origin coho when spawning in the wild. We used eight immune-relevant gene-linked microsatellite markers, four linked to immune-relevant expressed sequence tags (ESTs); and four linked to the Major Histocompatibility Complex (MHC). A three-generation pedigree study conducted on the Umpqua River, Oregon evaluated the relative RS of wild and hatchery-reared coho salmon in a wild setting. This pedigree offered a valuable opportunity to then assess mate choice by identifying mate pairs, their RS, and associated immune genetic diversity. The following classes of wild spawning mate pairs that occurred in 2005 and 2006 were evaluated: wild x wild, hatchery x hatchery, and wild x hatchery. In Chapter 2, we report on non-random associations of the number of shared immune alleles between each

mate pair for each of the three pair classes. In Chapter 3, we examine the relationship between mate pair RS and immune-relevant gene-linked marker diversity. Chapter 4 summarizes overall findings and considers their implications.

Chapter 2 – Evaluating Genetic Compatibility: Whether Coho Discriminate Immune-relevant Genotypes When Choosing Their Mates

2.1 Introduction

Mate preference is an important mechanism influencing offspring survival. It is based on evaluating a mate's qualities, using sensory cues, that may provide a selective advantage for their progeny. Genes that influence fitness play a direct role in mate choice because their diversity may affect survivorship. For example, a microsatellite polymorphism in the gamma interferon gene, identified in a quantitative trait locus mapping study, is associated with resistance to gastrointestinal nematodes in soay sheep (*Ovis aries*) (Coltman et al., 2001). The gamma interferon gene is involved in innate and adaptive immunity against viral and bacterial infections (Schoenborn and Wilson, 2007).

The MHC is an essential component of pathogen recognition and as a result MHC loci are under strong selective pressure (Klein, 1979; Potts et al., 1994). The MHC has been implicated in salmon mating preferences owing to the potential genetic benefits conferred to offspring (Landry et al., 2001; Consuegra and De Leaniz, 2008; Evans and Neff, 2009; Evans et al., 2012). There are two primary hypotheses involving MHC-mediated mate choice: (1) a dissimilar MHC mate choice prefer-

ence and (2) an intermediate MHC mate choice preference.

The dissimilar MHC-mediated mate preference hypothesis is based on the following logic: individuals who are more diverse at the MHC are thought to have a selective advantage since they should be able to recognize and make an immune response against a broader array of pathogens than individuals less diverse at the MHC (Hedrick, 1998; Bernatchez and Landry, 2003). Additionally, in species where males provide no post-spawning parental care or direct material benefits, females should prefer males that will increase the overall genetic quality and diversity of their progeny (Fleming, 1996). Several studies have demonstrated findings consistent with this hypothesis in Pacific salmon (Landry et al., 2001; Evans and Neff, 2009).

Roberts (2009) provided a review of the complexity of MHC-correlated mating preferences in wild populations and suggested that MHC-mediated mate choice is not always explained by choosing the most dissimilar mate, but rather a reflection of the trade-off between MHC dissimilarity and other desirable traits which serve to dilute the underlying dissimilarity preference. This alternative strategy represents an intermediate MHC-mediated mate choice preference. Several studies provide evidence for mate choice that favors an optimal level of MHC diversity (Reusch et al., 2001; Kalbe et al., 2009; Eizaguirre et al., 2009). Evans et al. (2012) documented a bet-hedging strategy in Atlantic salmon (*Salmo salar*), in which a trade-off exists between selection for offspring diversity and the unpredictable natural selection pressures imposed by parasites that will be faced by offspring.

Although knowledge about associations of allelic diversity to fitness-related

traits (such as disease resistance) in salmon has resulted predominately from studies of the MHC, many other immune-relevant genes also contribute to the complexity of immune responses, experience different selection pressures and thus likely affect offspring survival (Sommer et al., 2005). To our knowledge, only one other study has evaluated mate choice at non-MHC loci in fish (Jensen et al., 2007). The inclusion of immune-relevant genes in addition to MHC when evaluating mate choice enables a broader perspective on the functional relevance of alternate polymorphisms and may contribute to identification of other linkages or associations with fitness differences within and among populations of hatchery and wild salmon.

The aim of this study was to determine whether coho (*Oncorhynchus kisutch*) discriminate between immune-relevant genotypes when choosing their mates, and if they do, whether a dissimilar or intermediate preference is exhibited. Eight immune-relevant gene-linked microsatellites, four linked to immune-relevant expressed sequence tags (ESTs) and four linked to MHC coding regions, were used to evaluate the relative importance of mate choice in terms of non-random immune-relevant allelic associations. Previous studies utilizing non-MHC immune-relevant genes did not employ a hypothesis when evaluating mating strategies based on diversity at immune genes. However, as these genes are likely under the same selective pressures and affect offspring survival similarly, they were also evaluated for a dissimilar or intermediate preference.

2.2 Materials and Methods

2.2.1 Population and Reproductive Success Data

We obtained samples from a previous study that examined differences in reproductive success (RS) between wild and hatchery-reared coho salmon from the Umpqua River, in Southern Oregon, USA (Figure 2.1) (Thériault et al., 2011). Briefly, Oregon Department of Fish and Wildlife biologists collected wild and hatchery-reared coho from the North Umpqua River in 2001, 2002, and 2003 and created broodstock crosses of hatchery (H x H) and wild (W x W) fish in captivity for each year using single pair mating. The hatchery-reared coho were then released into the wild as unfed fry and smolts. All returning adults (2004 - 2006), including returns from natural wild matings, were sampled at a fish trap located at the base of Nonpareil Dam (Figure 2.1). After tissue samples were taken for genetic identification, all fish were released above the dam and allowed to spawn naturally. Subsequent adult offspring returns (2007 - 2009, F2 generation) were sampled and released in an identical fashion. Genotype data at 10 neutral microsatellite loci (*Ots519, Ots520, One111, P53, Ots3, Oneμ2, Ocl8, Ots215, ONEμ13, OMY1011*) were utilized to assign family pedigree using the software PAPA 2.0 and PASOS 1.0, and estimate individual RS (see Moyer et al. 2007; Thériault et al. 2010, 2011 for additional methodological details). In total, RS of the first two generations of coho was measured by reconstructing a three-generation pedigree. Coho only spawn once, so here individual RS is a measure of lifetime fitness; one generation from returning adult to returning adult.

For this study, we evaluated the 2005 and 2006 adult returns. Specifically, the three potential classes of wild spawning mating pairs were assessed: wild x wild (2005: n = 247; 2006: n = 188), hatchery x hatchery (2005: n = 222; 2006: n = 508), and wild x hatchery (2005: n = 333; 2006: n = 417) (Figure 2.2). Pairs will be hereafter referred to as follows: W x W (wild x wild), H x H (hatchery x hatchery), and W x H (wild x hatchery). In total, 1,561 individuals were evaluated since some individuals were involved in multiple mating classes. Mate pair RS is defined as the number of surviving adult offspring produced per mate pair. Jacks were excluded from all mate choice analyses.

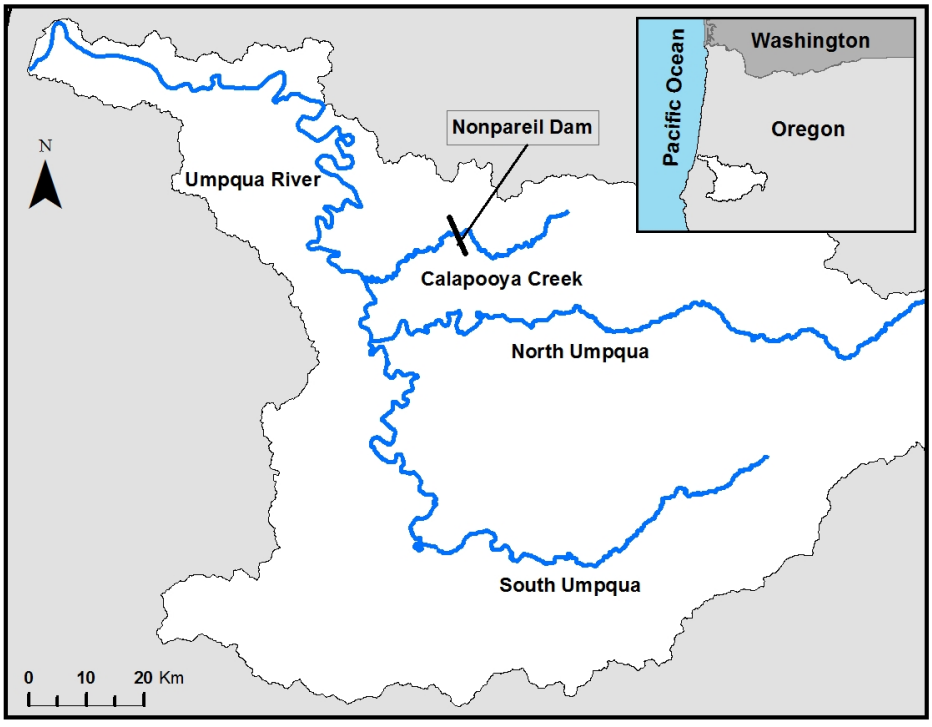

Figure 2.1: Map of the Umpqua River basin, Oregon USA. The fish trap located at Nonpareil Dam is highlighted in bold on Calapooya Creek.

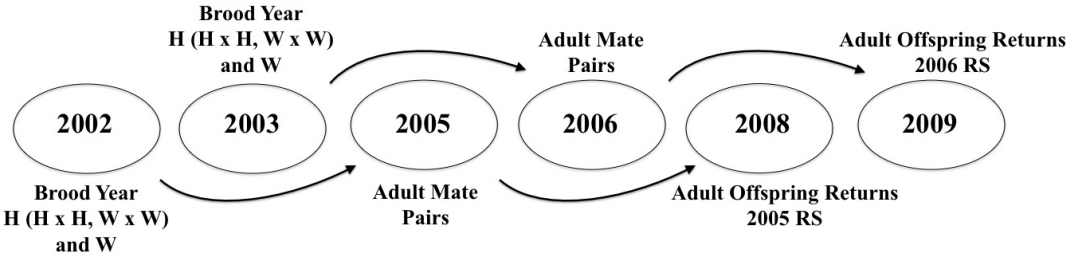

Figure 2.2: Generational schematic of wild spawning coho mate pairs (2005 and 2006) used in this study. The two brood years (2002 and 2003) indicate when crosses (H x H and W x W) were made in the hatchery and also when wild (W) returns were sampled.

2.2.2 Immune-relevant Gene-linked Marker Selection

A review of the literature provided three studies that have previously characterized Major Histocompatibility Complex (MHC)-linked microsatellites (Vasemägi et al., 2005; Johnson et al., 2008) as well as additional immune-relevant gene-linked microsatellites (Vasemägi et al., 2005; Tonteri et al., 2008) in salmon. Five additional MHC-linked microsatellites (*Oncorhynchus mykiss*) were provided from Caird Rexroad III (personal communication). Primer sets that could be optimized in coho using agarose gels were then genotyped to assess polymorphism in a 96 sample subset of both hatchery-reared and wild individuals (Table 2.1).

2.2.3 Genotyping

DNA from 2005 and 2006 returns contributing to the three classes of wild spawning mate pairs, W x W, H x H, and W x H, was previously extracted to assign parent-

Table 2.1: Immune-relevant gene-linked markers used to characterize coho mate pairs from the Umpqua River basin. NA_A refers to loci that could not be amplified in coho (PCR and agarose gel). NA_B refers to loci that could not be scored consistently in coho (ABI3730XL). EST-linked markers (Tonteri et al., 2008) that were identified as putative genes were discovered using the Basic Local Alignment Tool from the National Center for Biotechnology Information. Accession numbers are listed in parentheses.

Immune-relevant			MHC	
Locus	No. Alleles	Putative Gene	Locus	No. Alleles
SsalR001TKU*	2	–	*MHC1*$^+$	NA_B
SsalR002TKU*	NA_A	Barrier-to-autointegration factor (BT049316)	*OMM1189*$^\mp$	NA_A
SsalR003TKU*	2	Ubiquitin-fold modifier 1 (NM_001146524)	*OMM3024*$^\mp$	NA_A
SsalR004TKU*	NA_A	Import inner membrane translocase subunit (BT044803.1)	*OMM3026*$^\mp$	7
SsalR005TKU*	NA_A	DEAD box polypeptide 21 (BT044047.1)	*OMM3028*$^\mp$	2
SsalR006TKU*	2	–	*OMM3079*$^\mp$	1
SsalR007TKU*	1	Calpain small subunit 1(BT125513.1)	*OMM3080*$^\mp$	NA_A
SsalR008TKU*	1	–	*OMM3084*$^\mp$	2
SsalR009TKU*	NA_A	–	*OMM3089*$^\mp$	NA_B
SsalR010TKU*	15	Calmodulin (BT060158.1)	*BHMS429*$^\mp$	49
SsalR011TKU*	1	FYN-binding protein (NM_001140039.1)	*UBA3*$^\mp$	2
SsalR012TKU*	1	Leukocyte cell-derived chemotoxin 2 precursor (BT048115.2)	*OMM3025*$^\diamond$	NA_A
SsalR013TKU*	24	Filamin-A (ACN58728)	*OMM3027*$^\diamond$	2
SsalR014TKU*	2	Clusterin (NM_001173637.1)	*OMM3085*$^\diamond$	53
SsalR015TKU*	9	–	*OMM3090*$^\diamond$	NA_B
SsalR016TKU*	17	Vasodilator-stimulated phosphoprotein (NM_001140907)	*OMM3115*$^\diamond$	56
LEC$^+$	2			
IGF$^+$	2			
ARP$^+$	NA_B			

*Tonteri et al. (2008), $^+$Vasemägi et al. (2005), $^\mp$Johnson et al. (2008), $^\diamond$personal communication with Caird Rexroad III

age (Thériault et al., 2011) following the methods of Moyer et al. (2007). Primers for immune-relevant gene-linked markers that proved to be polymorphic in coho (*OMM3026, OMM3085, OMM3115, BHMS429, SsalR010TKU, SsalR013TKU, SsalR015TKU*, and *SsalR016TKU*, see Table 2.1 for details) were used to genotype all individuals (2005: n = 670; 2006: n = 891) involved in the pairs described above. PCR was performed separately in 5uL volumes incorporating fluorescently labeled forward primers according to the conditions recommended by the authors (see Vasemägi et al. 2005; Johnson et al. 2008; Tonteri et al. 2008 and Caird Rexroad III (personal communication)). PCR products were electrophoresed on an ABI 3730XL DNA Analyzer and scored as length polymorphisms using GeneMapper Software (Applied Biosystems, Inc., Carlsbad, CA).

2.2.4 Statistical Analyses - Approach 1

To test for a departure from random mating expectations, the number of shared alleles (none, one or two) between each mate pair was used as an estimator for genetic difference. This estimator was evaluated for a non-random association in two ways. The first approach allowed a comparison between successful pairs (RS $\geq$ 1) and all potential pairs that may have resulted in a mate pair RS of 0; while the second approach tested for a type of preference (dissimilar or intermediate), focusing only on successful pairs.

The first approach systematically paired every male and female involved in pairs with a RS $\geq$ 1 (2005: males = 317, females = 353; 2006: males = 414,

females = 477) to simulate all possible pairs. A pool of inferred mate pairs was then created by removing the observed pairs (successful pairs with a RS $\geq$ 1) from the generated simulated pair list. The average number of shared alleles at each immune-relevant marker was then compared between the observed and inferred mate pairs using a two-sample t-test. This approach was implemented for each class (W x W, H x H, and W x H) and year (2005 and 2006). To account for multiple testing, a false discovery rate correction was employed (Benjamini and Hochberg, 1995). All simulations and statistics were performed using R v. 2.13.2 statistical software (R Development Core Team, 2011).

2.2.5 Statistical Analyses - Approach 2

The second approach specifically evaluated what type of mate choice had occurred among observed mate pairs (RS $\geq$ 1); a dissimilar or intermediate preference. To address the dissimilarity preference, we calculated the mean genetic difference (MGD) between observed mate pairs at each immune-relevant marker. To test for an intermediate preference, we calculated the standard deviation genetic difference (SDGD) between observed mate pairs at each immune-relevant marker. Both statistics were then compared to expectations under random mating conditions by means of a permutation test. Individuals that contributed to the observed mate pairs (2005: W x W n = 247, H x H n = 222, W x H n = 333; 2006: W x W n = 188, H x H n = 508, W x H n = 417) were randomly paired (within sample) using equivalent n in each of 50,000 replicates to provide a non-choice comparison.

MGD and SDGD for actual mate pairs was then compared against the MGD and SDGD distribution of the 50,000 replicate random pairs. Standard deviation was used to evaluate an intermediate preference given that with such a preference, the observed MGD would be expected to be similar to the MGD of randomized pairs. W x W, H x H, and W x H mate pairs were evaluated separately for each year. False discovery rate corrections were employed (Benjamini and Hochberg, 1995) and R v. 2.13.2 statistical software was used for all analyses (R Development Core Team, 2011).

2.3 Results

2.3.1 Approach 1

We found no evidence for non-random mating in 2005 based on genetic difference at each of the eight immune-relevant gene-linked markers. Comparisons between the observed and inferred mate pairs within each of the three pair classes were non-significant (two-tailed two-sample t-tests, $p > 0.05$, Appendix Table A.1). Similarly, in 2006 there was no significant difference between observed and inferred W x W and H x H mate pairs based on genetic difference at each of the eight immune-relevant gene-linked markers (two-tailed two-sample t-tests, $p > 0.05$, Appendix Table A.1).

In contrast, the observed 2006 W x H mate pairs were significantly different from inferred pairs based on genetic difference at *BHMS429*, a marker linked to

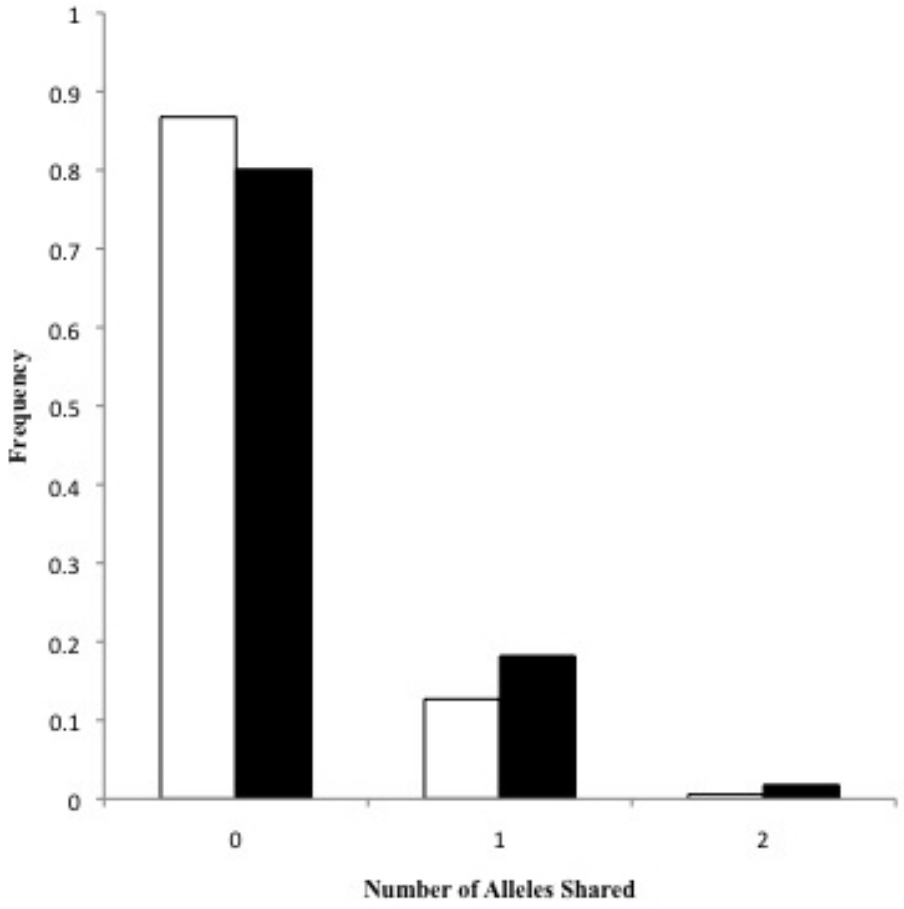

Figure 2.3: The frequency of shared alleles for observed (black columns) and inferred (white columns) 2006 wild x hatchery pairs at *BHMS429*. The frequency is standardized by the total number of pairs in each pair class.

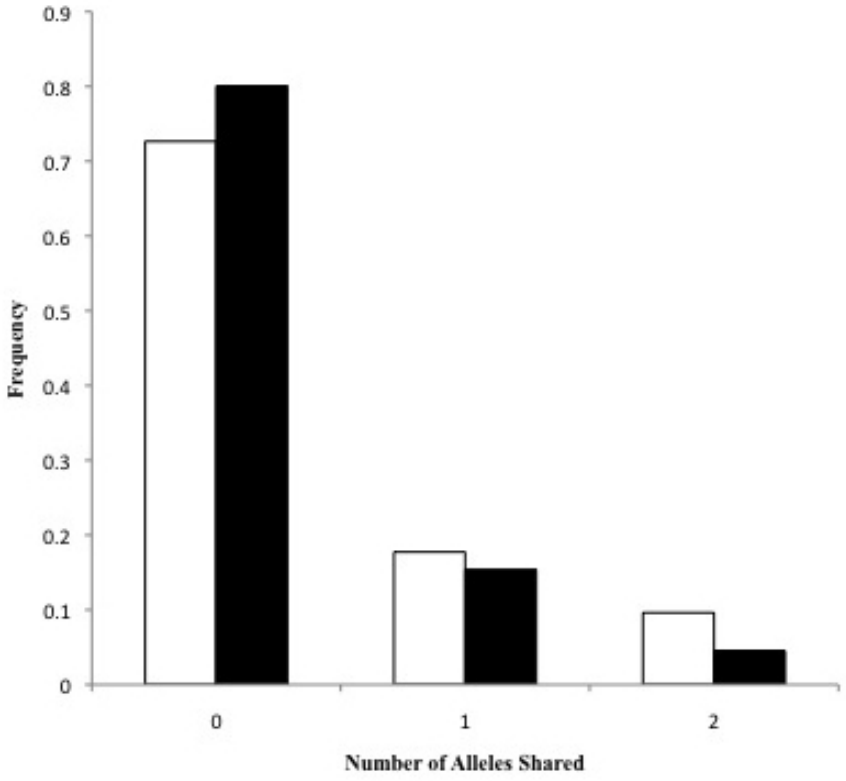

Figure 2.4: The frequency of shared alleles for observed (black columns) and inferred (white columns) 2006 wild x hatchery pairs at *SsalR013TKU*. The frequency is standardized by the total number of pairs in each pair class.

MHC class IB (two-tailed two-sample t-test, p = 0.0004, Appendix Table A.1), and at *SsalR013TKU*, an immune-relevant EST-linked marker (two-tailed two-sample t-test, p < 0.0005, Appendix Table A.1). There were fewer observed pairs that shared 0 alleles than inferred pairs at *BHMS429*, while there were more observed pairs that shared 0 alleles than inferred pairs at *SsalR013TKU* (Figures 2.3 and 2.4). There was no significance between 2006 W x H observed and inferred mate pairs based on genetic difference at the other six loci (*OMM3026, OMM3085, OMM3115, SsalR010TKU, SsalR015TKU, SsalR016TKU*, Appendix Table A.1). The ten neutral markers used to construct the genetic pedigree (see Section 2.2.1) were also evaluated using this approach and no significant results were found within each of the three pair classes in either year (See Appendix Table B.1).

2.3.2 Approach 2

2.3.2.1 Results for 2005

We found no evidence for non-random dissimilar mating based on mean genetic difference (MGD) in 2005. At all loci (*OMM3026, OMM3085, OMM3115, BHMS429, SsaIR010TKU, SsaIR013TKU, SsalR015TKU,* and *SsaIR016TKU*), the observed MGD was not significantly different from the MGD distribution of replicate random W x W, H x H, and W x H mate pairs (two-tailed permutation tests, p > 0.05, Figures 2.5 to 2.7 and Appendix Table A.2). The neutral markers used to assign parentage were also evaluated for departures from random expectations for

MGD and were not significant at any of the pair classes (see Appendix Table B.2).

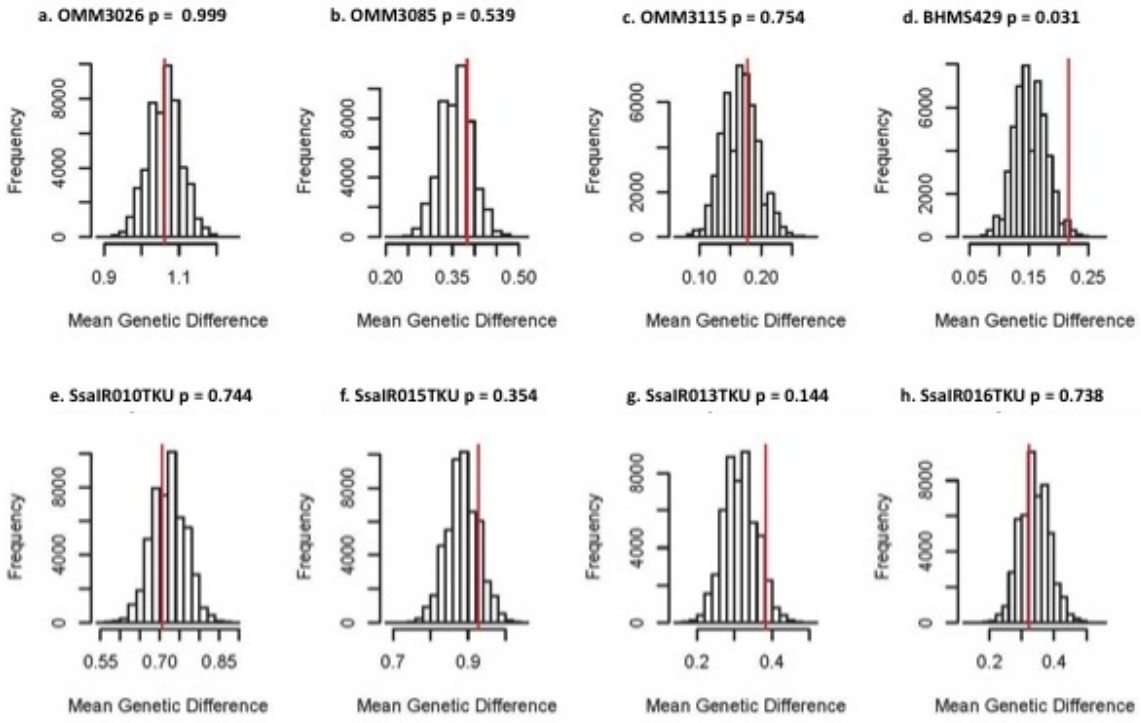

Figure 2.5: Observed mean genetic difference (red line) of 2005 wild x wild mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

We did find evidence for non-random intermediate mating based on standard deviation genetic difference (SDGD) in 2005 H x H mate pairs, where the observed SDGD was exceeded by the SDGD distribution of replicate random pairings at *SsaIR015TKU*, an immune-relevant EST-linked marker (one-tailed permutation test, p = 0.003, Figure 2.9f). The other seven loci (*OMM3026, OMM3085, OMM3115, BHMS429, SsaIR010TKU, SsaIR013TKU,* and *SsaIR016TKU*) were

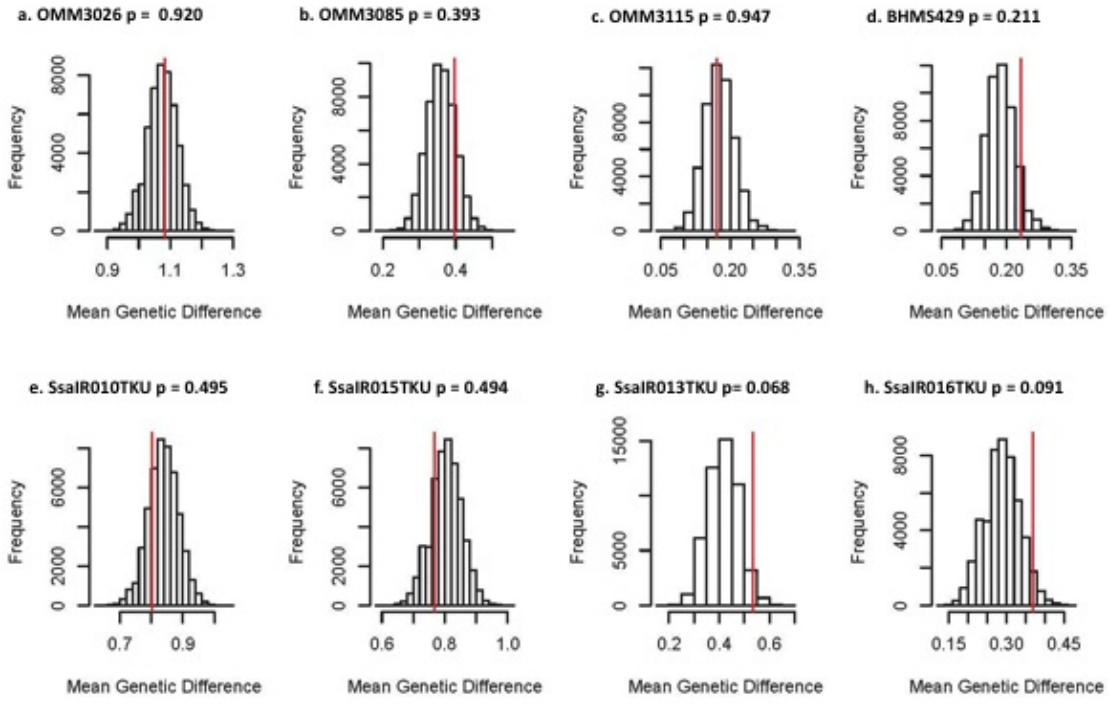

Figure 2.6: Observed mean genetic difference (red line) of 2005 hatchery x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

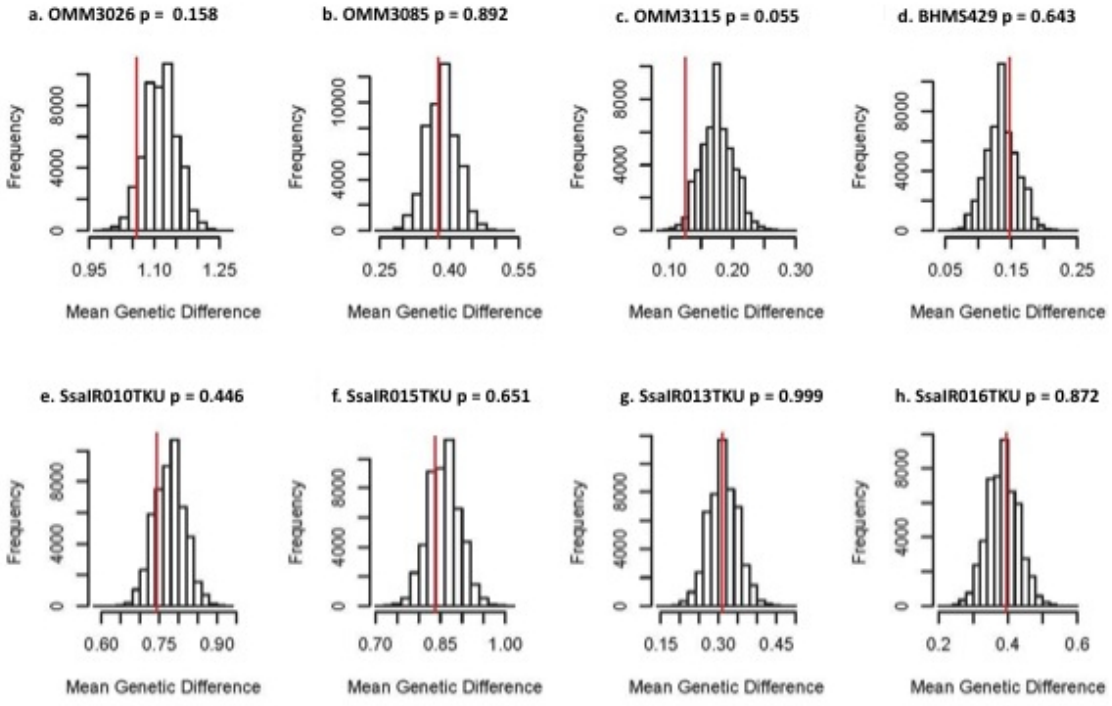

Figure 2.7: Observed mean genetic difference (red line) of 2005 wild x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

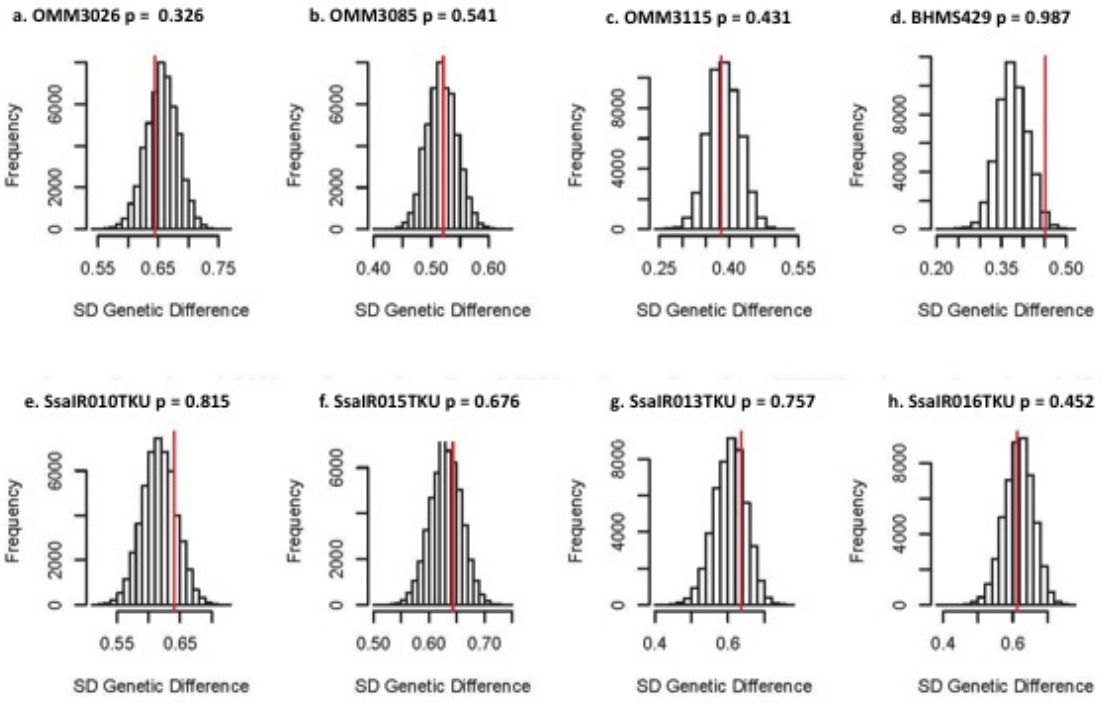

Figure 2.8: Observed standard deviation genetic difference (red line) of 2005 wild x wild mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

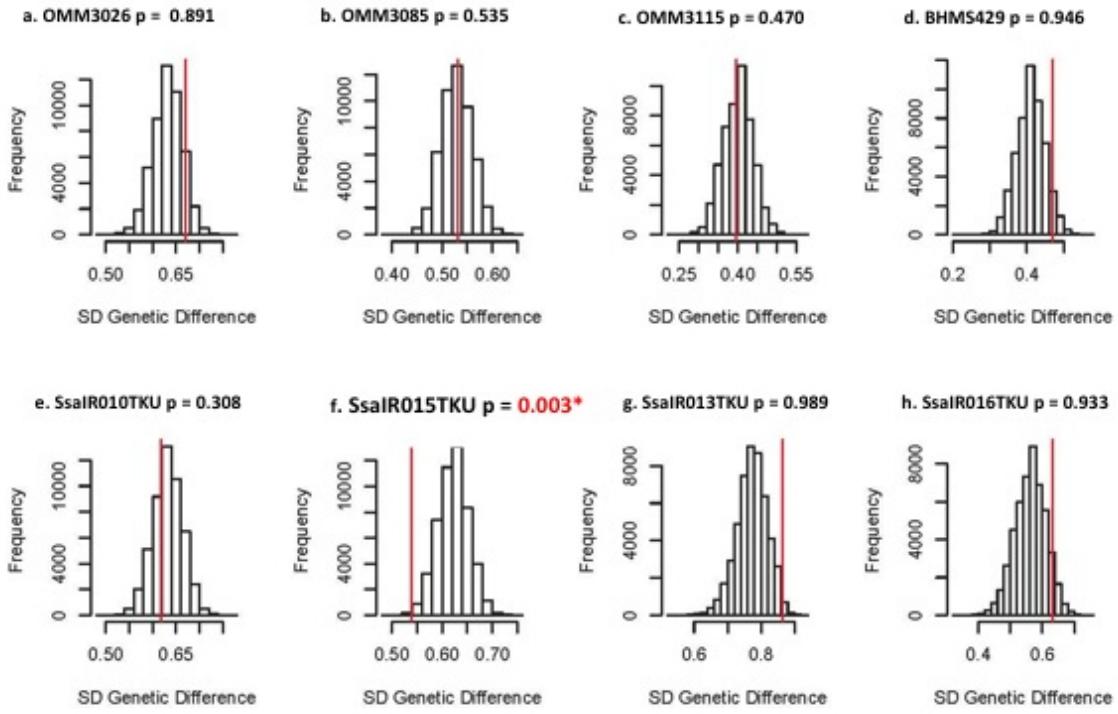

Figure 2.9: Observed standard deviation genetic difference (red line) of 2005 hatchery x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing (*SsalR015TKU*).

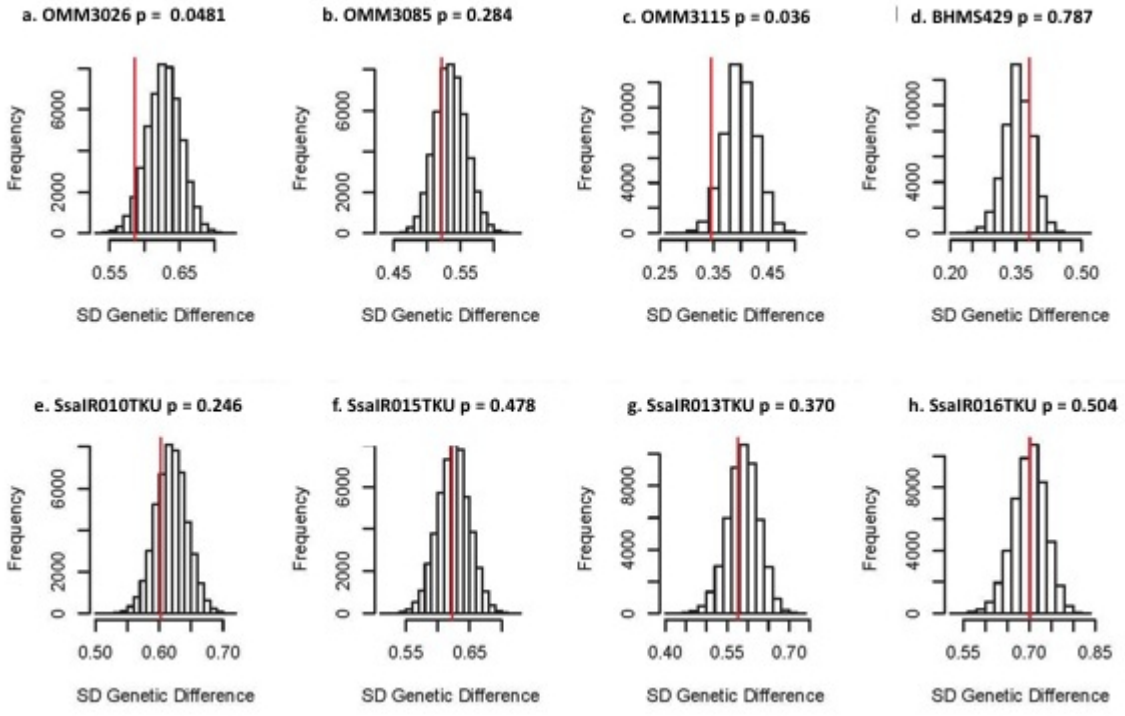

Figure 2.10: Observed standard deviation genetic difference (red line) of 2005 wild x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

not significant (one-tailed permutation tests, $p > 0.05$, Figure 2.9, Appendix Table A.3).

We found no evidence for non-random intermediate mating based on SDGD in 2005 W x W or W x H mate pairs. Observed SDGD did not differ significantly from random expectations at all loci (*OMM3026, OMM3085, OMM3115, BHMS429, SsaIR010TKU, SsaIR013TKU, SsaIR015TKU* and *SsaIR016TKU*, one-tailed permutation tests, $p > 0.05$, Figures 2.8 and 2.10, Appendix A.3). The neutral markers used to assign parentage were evaluated for departures from random expectations for SDGD and displayed no significance at any of the mating pair classes (see Appendix Table B.3).

2.3.2.2 Results for 2006

We found evidence for non-random mating based on mean genetic difference (MGD) in 2006. W x H mate pairs demonstrated a significant departure from random expectations for MGD at *BHMS429* (two-tailed permutation test, $p = 0.001$, Figure 2.13d) and *SsalR016TKU* (two-tailed permutation test, $p = 0.01$, Figure 2.13h). W x H observed MGD exceeded the randomized MGD distribution at *BHMS429*, an MHC-linked marker, thereby exhibiting a similarity mate preference (Figure 2.13d). In contrast, the W x H observed MGD was exceeded by the MGD distribution of randomized pairs at *SsalR016TKU*, an immune-relevant expressed sequence tag (EST)-linked marker, demonstrating a dissimilarity mate preference (Figure 2.13h). The other six loci (*OMM3026, OMM3085, OMM3115,*

SsalR010TKU, SsalR013TKU, and *SsalR015TKU*) were non-significant in terms of the observed and replicate random pair MGD distribution (two-tailed permutation tests, $p > 0.05$, Figure 2.13, Appendix Table A.2).

We did not find evidence for non-random mating based on MGD in 2006 W x W or H x H mate pairs. At all loci, (*OMM3026, OMM3085, OMM3115, BHMS429, SsaIR010TKU, SsaIR013TKU, SsaIR015TKU* and *SsaIR016TKU*) observed MGD did not significantly differ from random expectations (two-tailed permutation tests, $p > 0.05$, Figures 2.11 and 2.12, Appendix Table A.2). The neutral markers used to assign parentage were also evaluated for departures from random expectations for MGD and were not significant at any of the pair classes (see Appendix Table B.2).

We found evidence for non-random intermediate mating based on standard deviation genetic difference (SDGD) in 2006. W x H mate pair observed SDGD was exceeded by the randomized pair distribution of SDGD at *SsalR016TKU*, an immune-relevant expressed sequence tag (EST)-linked marker (one-tailed permutation test, $p = 0.002$, Figure 2.16h). The remaining seven loci (*OMM3026, OMM3085, OMM3115, BHMS429, SsaIR010TKU, SsaIR013TKU,* and *SsaIR016TKU*) were not significant (one-tailed permutation tests, $p > 0.05$, Figure 2.16, Appendix Table A.3).

We did not find evidence for non-random intermediate mating based on SDGD in 2006 W x W and H x H mate pairs. At all loci (*OMM3026, OMM3085, OMM3115, BHMS429, SsaIR010TKU, SsaIR013TKU, SsalR015TKU,* and *SsaIR016TKU*), the observed SDGD was not significantly different from the distribution of replicate

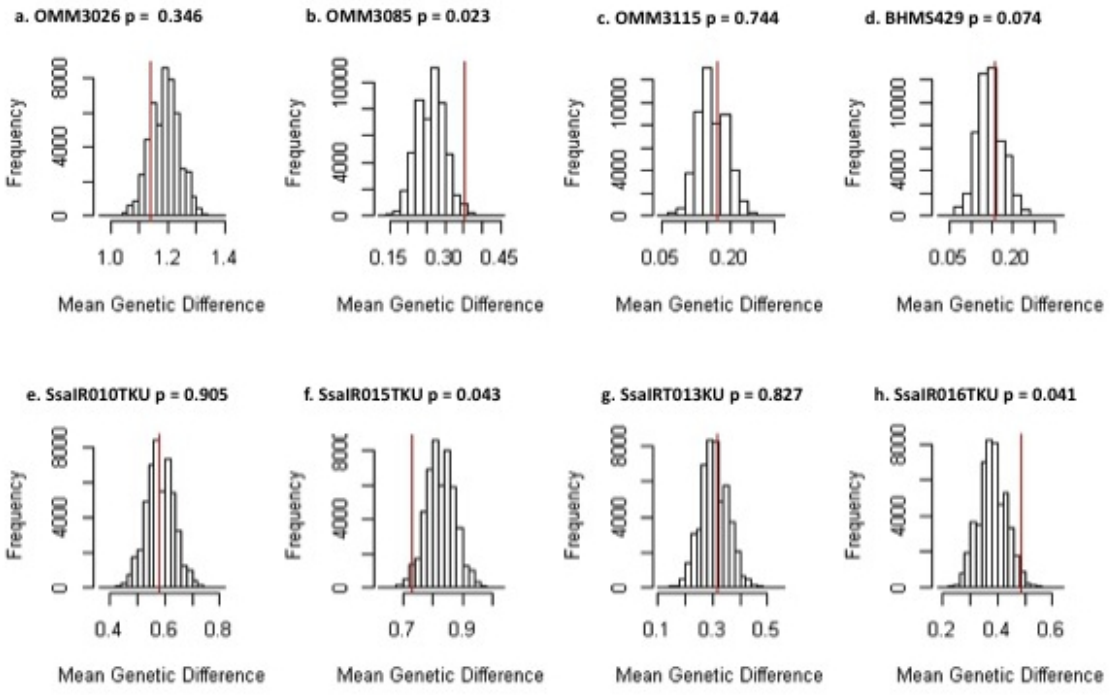

Figure 2.11: Observed mean genetic difference (red line) of 2006 wild x wild mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

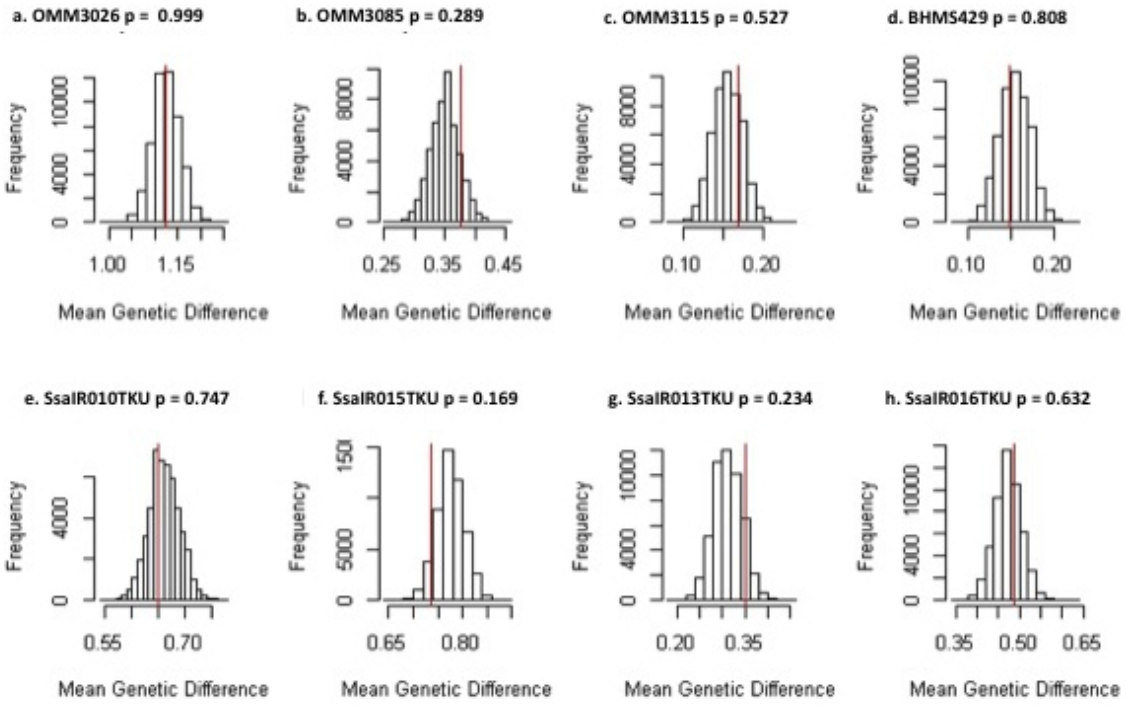

Figure 2.12: Observed mean genetic difference (red line) of 2006 hatchery x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

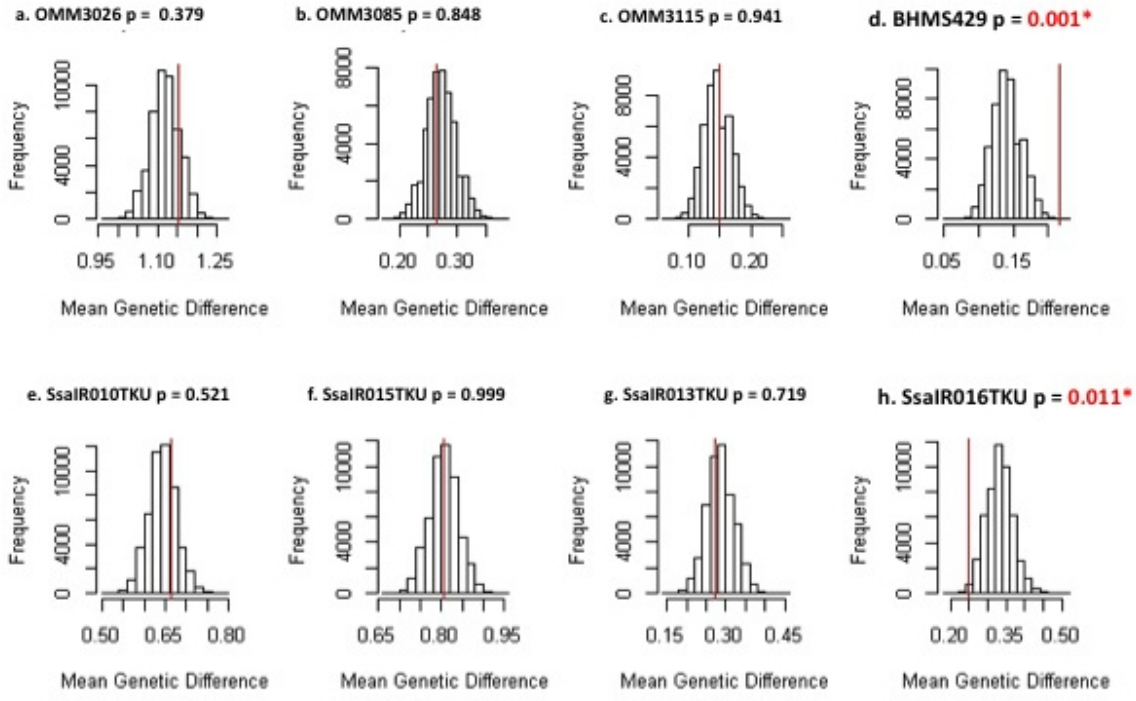

Figure 2.13: Observed mean genetic difference (red line) of 2006 wild x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing (*BHMS429* and *SsalR016TKU*).

random pairs (one-tailed permutation tests, $p > 0.05$, Figures 2.14 and 2.15, Appendix Table A.3). The neutral markers used to assign parentage were evaluated for departures from random expectations for SDGD and displayed no significance at any of the mating pair classes (see Appendix Table B.3).

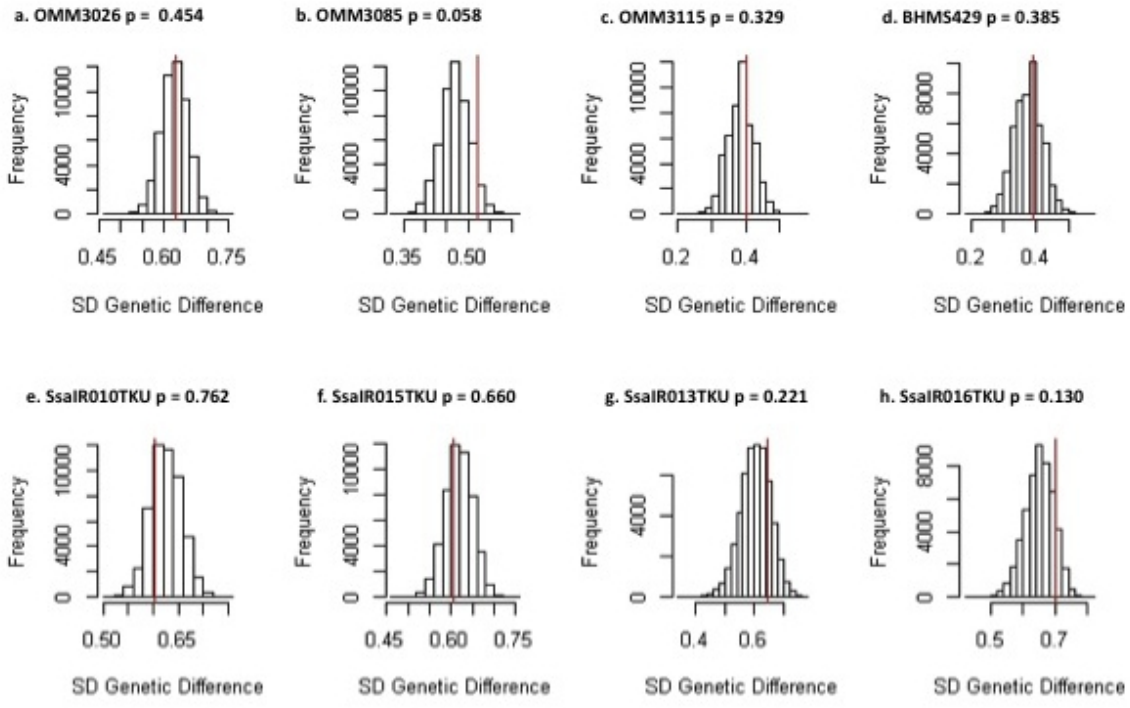

Figure 2.14: Observed standard deviation genetic difference (red line) of 2006 wild x wild mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

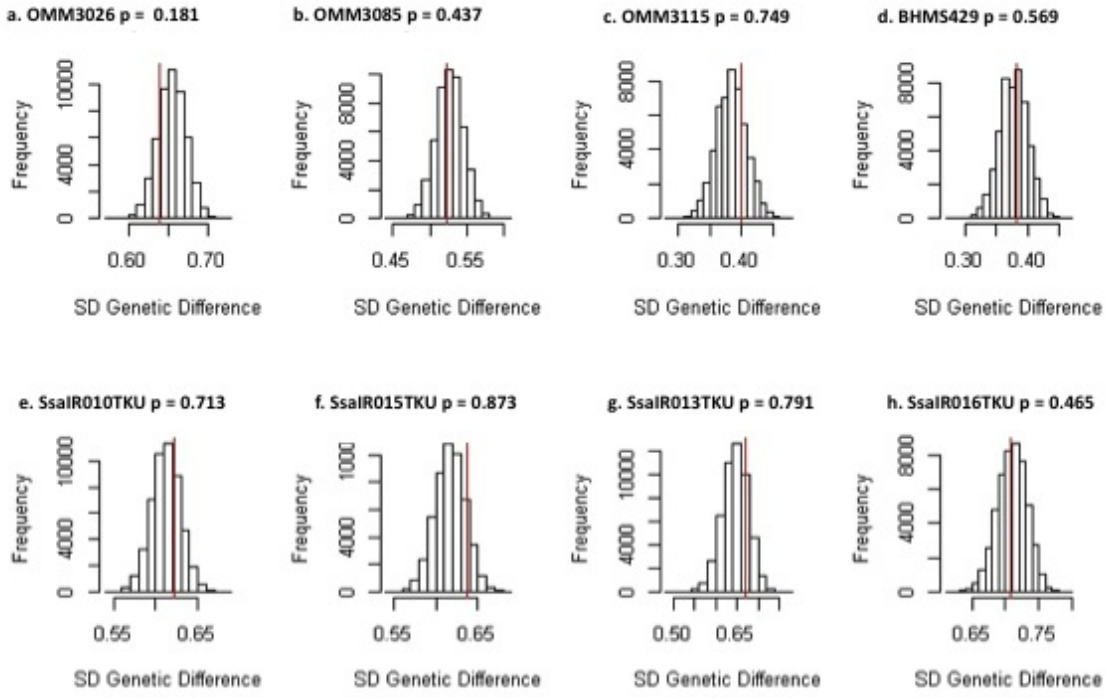

Figure 2.15: Observed standard deviation genetic difference (red line) of 2006 hatchery x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing.

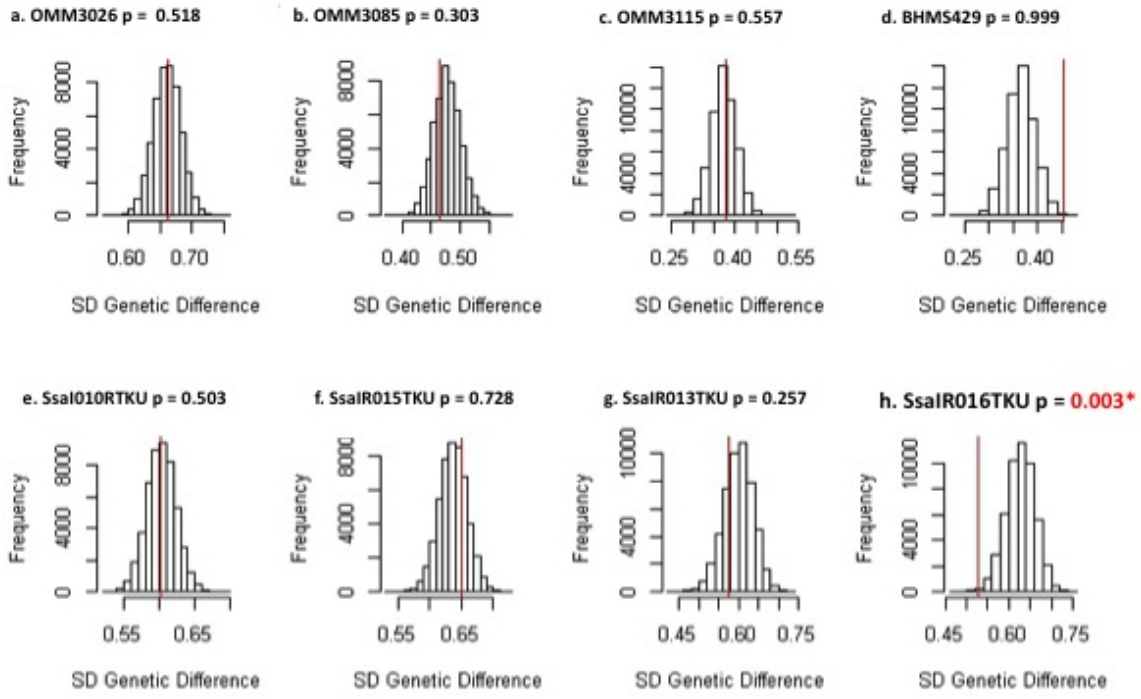

Figure 2.16: Observed standard deviation genetic difference (red line) of 2006 wild x hatchery mate pairs compared with expected results from random mating (50,000 replicates). Markers with an asterisk indicate those that remained significant after false discovery rate correction for multiple testing (*SsalR015TKU*).

2.4 Discussion

Previous studies examining mate choice in fish have mostly focused on patterns between mate pairs at the Major Histocompatibility Complex (MHC) (Milinski, 2006). Here, we extended the approach developed at the MHC level to test whether a non-random mate choice signature could be detected at additional immune-relevant gene-linked markers. Given the wealth of MHC-dependent fish mate choice studies (Arkush et al., 2002; Kurtz et al., 2004; Aguilar and Garza, 2006; De Eyto et al., 2007; Dionne et al., 2007; Evans et al., 2012) and even one non-MHC immune-mediated fish mate choice study (Jensen et al., 2007), it was conceivable that we might see some type of mate choice signature based on immune-relevant gene-linked markers selected in this study. We found evidence for non-random mating, but our results were not consistent across years. In 2006, there was evidence for non-random mating between W x H mate pairs across Approach 1 and Approach 2. In contrast, we did not find evidence for non-random mating in Approach 1 or when assessing a dissimilarity preference in Approach 2 for all pair classes in 2005. However, 2005 H x H mate pairs had a significant intermediate preference (Approach 2) at one immune-relevant marker.

The most salient result of this study was the statistically significant support for non-random allelic associations between 2006 W x H mate pairs in evaluating the type of preference (Approach 2: mean genetic difference (MGD) and standard deviation genetic difference (SDGD)) as well as a general departure from random expectations (Approach 1). *SsalR016TKU*, an immune-relevant expressed

sequence tag (EST)-linked marker, displayed both a significant dissimilarity preference and a significant intermediate preference. Additionally, the locus *BHMS429*, an MHC-linked marker, exhibited a similarity preference in terms of the observed and replicate random MGD distribution.

2006 W x H observed pairs at *BHMS429* were significantly different from inferred pairs in Approach 1 as well. Moreover, while Approach 1 didn't evaluate the specific type of mate choice, Figure 2.3 is also indicative of a similarity preference at *BHMS429*. The observed pairs had a lower frequency of pairs that shared 0 alleles and a higher frequency of pairs that shared 1 and 2 alleles when compared to the inferred pairs. Nevertheless, it should be noted that the expected heterozygosity was greater than 0.9 for hatchery-reared and wild fish from both years at this locus so it is likely that the generation of all potential pairs in Approach 1 and randomized pairs in Approach 2 would have produced mostly pairs that shared zero alleles, allowing little power to demonstrate an even greater dissimilarity (See Appendix Table C.1). Additionally, *SsalR013TKU*, an immune-relevant expressed sequence tag (EST)-linked marker, was suggestive of a dissimilarity preference for 2006 W x H mate pairs in Approach 1. In this case, Figure 2.4 illustrates a higher frequency of observed pairs that shared 0 alleles and a lower frequency that shared 1 and 2 alleles when compared to inferred pairs.

A recent quantitative trait loci (QTL) mapping study found that *BHMS429* is linked to MHC class IB, 0.5 centimorgans separate them, in rainbow trout (*Oncorhynchus mykiss*) (Rexroad et al., 2005). Putative gene identification was evaluated for both EST-linked markers, (*SsalR013TKU* and *SsalR016TKU*), using

the Basic Local Alignment Tool from the National Center for Biotechnology Information (NCBI). *SsalR013TKU* had a 99% identity to the actin binding protein Filamin-A in Atlantic salmon (Accession number: ACN58728). One function of Filamin-A is to link actin to caveolae, which are membrane invaginations that mediate viral entry (Muriel et al., 2011). *SsalR016TKU* had a 99% identity to a vasodilator-stimulated phosphoprotein in Atlantic salmon (Accession number: NM_001140907, Leong et al. 2010). Two of the roles of vasodilator-stimulated phosphoproteins are to regulate actin dynamics in platelets and regulate platelet aggregation (Li Calzi et al., 2008).

We did not find evidence for non-random association in any of the pair classes (W x W, H x H, and W x H) for 2005 mate pairs when comparing observed to inferred pairs (Approach 1) and when assessing a dissimilarity preference (Approach 2). These results illustrate one of the difficulties associated with studying mate choice *in situ*; mate preferences are estimated subsequently (Forsberg et al., 2007) and may be masked by other desirable traits such as male size (Petersson et al., 1999; Eizaguirre et al., 2009) or competitive behavior (i.e. male aggression, Casalini et al. 2009; Garner et al. 2010). Patterns of mating in wild salmonid populations are complex and therefore not easy to predict (Quinn, 2005; Roberts et al., 2006). It may also be the case that by using immune-relevant gene-linked microsatellites rather than assessing allelic variances and associations at the actual genes themselves, significant mate choice signals were not detected due to inconsistent linkage between immune genes and microsatellite polymorphisms.

We did find a significant intermediate preference for 2005 H x H pairs at

SsalR015TKU, an immune-relevant expressed sequence tag (EST)-linked marker, when assessing an intermediate preference from Approach 2. This may be a result of reduced allelic variance in the hatchery-reared mate pairs, although this would not explain why none of the other loci exhibited a significant preference for 2005 H x H pairs. Putative gene identification was evaluated for this EST-linked marker and no significant similarity was found with the NCBI resource or by Tonteri et al. (2008). Consequently, no role in immune response can be linked to the 2005 H x H intermediate mate preference.

As noted by Bollmer et al. (2011) there is evidence for a range of selective forces on immunity genes, including positive and balancing selection, yet no clear pattern is evident, even in model organisms. The findings of this study, however, suggest that the observed high mating frequency of 2006 W x H pairs with significant departures from random expectations at immune-relevant linked markers is not a chance occurrence and provides evidence of a mate choice signal. There are three factors that may explain why 2006 W x H mate pairs were the only pair class to display a consistent mate choice signal across approaches. The first hypothesis that may have relevance to this phenomenon is differences in mate pair reproductive success (RS) between the three pair classes for each year. W x H mate pairs had the highest RS for both years (see Appendix Figure D.1). If mate pair RS is a product of mate choice, then W x H pairs would be most likely to exhibit a mate choice signal.

Secondly, disparities in mate choice signatures between years may also have been affected by differences in jack composition. Jacks are sexually mature males

that return to the spawning grounds a year earlier than their male counterparts, which mature at age 3 (Gross, 1985). As a result, jacks are much smaller, cannot compete for locations near females and instead use a sneaker strategy. In 2005, there were 70 jacks that were reproductively successful (RS $\geq$ 1) as opposed to 45 in 2006 (Thériault et al., 2011). Although the total number of mate pairs involving a jack was not that different between years (2005 = 127 mate pairs, 2006 = 122 mate pairs) (Thériault et al., 2011), there were more jacks with a RS $\geq$ 1 in 2006 than 2005 and more instances of multiple mating events (see Appendix Figure E.1 and Appendix Figure E.2). One could argue that this points towards a greater jack presence in 2005 than in 2006. If this is the case, jack mating events may explain why a significant mate choice signal was not exhibited in 2005.

Finally, overall density of returns might also be a factor that could affect mate choice signal in the wild. In 2005, 1,659 individuals were passed over Nonpareil Dam compared to 1,442 individuals in 2006. There is approximately 51.5 river km of coho habitat above Nonpareil Dam according to a 2008 seeding survey report (personal communication with Laura Jackson, Umpqua District Fish Biologist, Oregon Department of Fish and Wildlife). Christie et al. (2012a) documented an absence of domestic selection for hatchery-reared smolts in low-density conditions while selective pressure was present in high-density conditions. It is possible that similar density constraints on the spawning grounds may also affect mate choice decisions. In the current study, a lower overall density of fish occurred in 2006, which may be the reason why a mate choice signal was detected in 2006 and not in 2005.

Non-random mating is the likely explanation for the significant departures from random expectations observed in 2006 W x H mate pairs. However, all studied mate pairs had at least one offspring that returned as an adult to spawn. Thus, the observed patterns could reflect a combination of precopulatory (mate choice) and postcopulatory selection since only mate pairs that had successful spawning offspring were included in these analyses (Laurent et al., 2012).

Although, we couldn't demonstrate mate choice based on immune-relevant loci across all pair classes or years, it is still important to determine whether RS is associated with immune diversity. In other words, it is possible mating was random for the majority of mate pairs, but that matings which involved greater combinations of immune alleles produced more offspring that returned as adults. We attempt to address this question in Chapter 3. Such results warrant further examination among wild spawning salmonid populations in order to facilitate a fuller understanding of mate choice and associated fitness consequences; this is especially true in terms of hatchery supplementation implications.

Chapter 3 – The Relationship Between Immune-relevant Gene Diversity and Reproductive Success Among Wild Spawning Coho Salmon Mate Pairs

3.1 Introduction

Mate choice is based on assessing a potential mate's qualities to provide a selective advantage to offspring thereby increasing the reproductive success (RS) of the mate pair. Genes that influence fitness play a direct role in mate choice because their diversity can affect the survivorship of progeny. For instance, the Major Histocompatibility Complex (MHC) and additional immune-relevant genes all contribute to the complexity of immune responses. These genes experience strong selection pressures, affect offspring survival, and are therefore thought to be involved in mate selection.

In order to estimate the benefits of mate choice, offspring survival must be examined since there are no unequivocal surrogates for the genetic quality of individuals or the genetic compatibility of parents (Puurtinen et al., 2009). The concept of genetic compatibility rests on the idea that offspring survival can be increased by specific combinations of parental alleles. Thus, mate compatibility can yield a high genetic value for parent fitness, demonstrating the advantage of mate selection. Many studies have only measured juvenile (less than 1 year

old) offspring survival when evaluating mate choice based on immune gene diversity (Landry et al., 2001; Forsberg et al., 2007; Garner et al., 2010; Neff et al., 2008). To fully assess the genetic benefits of mate preference, lifetime reproductive consequences must be measured given that passing on one's genetic material is the ultimate goal (Kalbe et al., 2009). A powerful aspect of this study is that lifetime RS (one generation from returning adult to returning adult) was evaluated.

In this study, we hypothesized that diversity at immune-relevant genes is associated with mate pair RS. We tested this by assessing whether the number of shared alleles at a suite of immune-relevant genes between wild spawning coho salmon (*Oncorhynchus kisutch*) mate pairs is correlated to lifetime RS. Eight immune-relevant gene-linked microsatellites including four linked to immune-relevant expressed sequence tags (ESTs) and four linked to MHC coding regions were employed. In addition, we evaluated four variables that might also influence RS: fork length, run date, origin of the individuals involved in each pairing, and run date difference between the male and female.

3.2 Materials and Methods

3.2.1 Population and Reproductive Success Data

Mate pairs used in this study were identical to those described in Section 2.2.1. To review, pairs and their respective reproductive success (RS) were identified from a previous study that constructed a three-generation pedigree of wild and

hatchery-reared coho salmon from the North Umpqua River, in Southern Oregon, USA (Figure 2.1, see Moyer et al. 2007; Thériault et al. 2010, 2011 for additional methodological details). Mate pair RS is defined as the number of surviving adult offspring produced per mate pair (lifetime RS). We evaluated three classes of wild spawning mate pairs in two different years: wild x wild (2005: n = 247; 2006: n = 188), hatchery x hatchery (2005: n = 222; 2006: n = 508), and wild x hatchery (2005: n = 333; 2006: n = 417) (Figures 2.2 and 3.1). Pairs will hereafter be referred to as: W x W (wild x wild), H x H (hatchery x hatchery), and W x H (wild x hatchery).

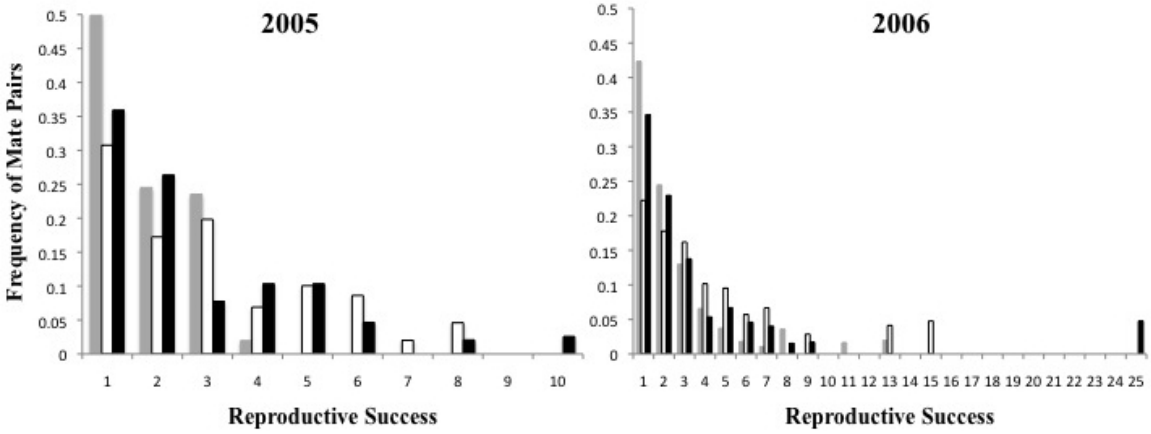

Figure 3.1: Histograms of mate pair reproductive success for 2005 and 2006 coho returns. The frequency is standardized by the total number of mate pairs in each pair class. Gray columns = hatchery x hatchery mate pairs, white columns = wild x wild mate pairs, and black columns are wild x hatchery mate pairs.

3.2.2 Explanatory Variable Selection

We used a Poisson distributed generalized linear model (GLM) to determine whether immune gene diversity correlates to mate pair reproductive success (RS). The numbers of shared alleles between a mate pair at eight immune-relevant gene-linked markers (described in Section 2.2.2, see Table 2.1) were used as explanatory variables. The number of shared alleles between each mate pair was calculated as described in Section 2.2.4. In addition, fork length (mm), run date (Julian day), and origin (hatchery-reared or wild) of the male and female involved in each pairing, as well as the run date difference between the male and female were included as predictors of RS (Moyer et al., 2007; Thériault et al., 2010, 2011). These variables were incorporated as complementary covariates since they have been shown to affect individual RS (Petersson et al., 1999; Quinn, 2005; Thériault et al., 2011). The 15 explanatory variables are defined in Table 3.1.

3.2.3 Model Selection

Separate models were built for each mating class (W x W, H x H, and W x H) within each year (2005 and 2006) totaling six models. We assessed collinearity of explanatory terms to avoid fitting models with linear relationships between independent variables. Each explanatory variable, as well as all potential two-way interaction terms, were first evaluated individually for a significant predictive power of mate pair reproductive success (RS) prior to its inclusion in a saturated model. If an explanatory variable did not significantly predict variance in mate pair RS it was

Table 3.1: Covariates estimated for their effects on mate pair reproductive success variation.

Covariate	Definition
SsalR010TKU	number of shared alleles (0, 1, or 2) at this immune-linked marker
SsalR013TKU	number of shared alleles (0, 1, or 2) at this immune-linked marker
SsalR015TKU	number of shared alleles (0, 1, or 2) at this immune-linked marker
SsalR016TKU	number of shared alleles (0, 1, or 2) at this immune-linked marker
OMM3026	number of shared alleles (0, 1, or 2) at this MHC-linked marker
OMM3085	number of shared alleles (0, 1, or 2) at this MHC-linked marker
OMM3115	number of shared alleles (0, 1, or 2) at this MHC-linked marker
BHMS429	number of shared alleles (0, 1, or 2) at this MHC-linked marker
Female fork length	length of the female involved in the mate pairing (mm)
Male fork length	length of the male involved in the mate pairing (mm)
Female run date	date the female was passed above Nonpareil Dam to spawn (Julian day)
Male run date	date the male was passed above Nonpareil Dam to spawn (Julian day)
Female origin	origin of the female involved in the pairing (hatchery-reared or wild)
Male origin	origin of the male involved in the pairing (hatchery-reared or wild)
Run date difference	daily difference between female and male run date (Julian day)

excluded from further analyses. Those variables that were significant predictors of mate pair RS variance were then included in a saturated model. We used Akaikes information criterion (AIC) model selection to determine the combination of explanatory variables that best explained mate pair RS based on the lowest AIC value (Burnham and Anderson, 2002). We evaluated deviance residuals and tested for goodness-of-fit (Lehmann, 1975) to evaluate overdispersion for each model. Robust standard errors were calculated for all final model parameter estimates to control for mild violation of the distribution assumption that the variance equals the mean (Cameron and Trivedi, 2009). Analyses were performed using R v. 2.13.2 statistical software (R Development Core Team, 2011)

3.3 Results

3.3.1 Factors Influencing Mate Pair Reproductive Success

3.3.1.1 Wild x Wild Mate Pairs

The model that best predicted variance in 2005 W x W mate pair reproductive success (RS) consisted of the explanatory variables *BHMS429*, an MHC-linked marker, and female run date (Figure 3.2, Table 3.2). Our result supports the hypothesis that the number of shared alleles at immune-relevant gene-linked markers is associated with mate pair RS after accounting for additional variables that may influence mate pairing. There was a small difference between the deviance of our model and the maximum deviance of the ideal model (predicted values are identical

to the observed), providing no evidence of overdispersion. A goodness-of-fit chi-squared test was not significant and indicated that our model fit the data well ($p = 0.91$). There was no collinearity (Pearsons $r^2 < 0.40$) between the variables used in the final model. Female run date was correlated with male run date (Pearsons $r^2 = 0.51$) but male run date was not included in the final model. Furthermore, male run date did not have a significant relationship with mate pair RS when evaluated separately and when included in a model with all parameters. When keeping the other explanatory variable in the model constant, mean mate pair RS decreased by 23% for each allele increase in the number of alleles shared between mate pairs at *BHMS429* ($p = 0.01$, 95% C.I. = 37-6%). A 1-day (Julian day) increase in female run date resulted in a 0.08% increase in mean mate pair RS ($p = 0.04$, 95% C.I. = 0.002-2%; Table 3.2).

The model that best predicted variance in 2006 W x W mate pair RS consisted of the explanatory variables *BHMS429* and male run date (Figure 3.2, Table 3.2). This result supports our hypothesis that the number of shared alleles at immune-relevant gene-linked markers is associated with mate pair RS after accounting for additional variables that may influence mate pairing. There was a small difference between the deviance of our model and the maximum deviance of the ideal model, providing no evidence of overdispersion. A goodness-of-fit chi-squared test was not significant and indicated that our model fit the data reasonably well ($p = 0.58$). There was no collinearity (Pearsons $r^2 < 0.40$) between the variables used in the final model. When keeping the other explanatory variable in the model constant, mean mate pair RS decreased by 23% for each allele increase in the number of

alleles shared at *BHMS429* between mate pairs (p = 0.05, 95% C.I. = 39-0.01%). A 1-day (Julian day) increase in male run date resulted in a 1% increase in mean mate pair RS (p = 0.04, 95% C.I. = 0.06-2.6%; Table 3.2).

3.3.1.2 Hatchery x Hatchery Mate Pairs

For both 2005 and 2006 H x H mate pairs, there were no explanatory variables that significantly predicted mate pair reproductive success (RS). Therefore, our result does not support any relationship between number of shared alleles at immune-relevant gene-linked markers and mate pair RS for H x H mate pairs after accounting for additional variables that may influence mate pairing.

3.3.1.3 Wild x Hatchery Mate Pairs

The model that best predicted variance in 2005 W x H mate pair reproductive success (RS) included the explanatory variables female fork length, male run date, and run date difference (Figure 3.3, Table 3.3). Our results do not support the hypothesis that the number of shared alleles at immune-relevant gene-linked markers is associated with mate pair RS after accounting for additional variables that may influence mate pairing. There was a small difference between the deviance of our model and the maximum deviance of the ideal model, demonstrating no evidence of overdispersion. A goodness-of-fit chi-squared test was not significant and indicated that our model fit the data well (p = 0.99). There was no collinearity (Pearsons r^2

Table 3.2: Final poison log-linear regression models, determined by AIC model selection, of variables associated with 2005 and 2006 wild x wild mate pair reproductive success.

Year	Variable	Coefficient	Standard error	z-statistic	Two-sided P value	95% C.I.
2005	intercept	-19100	9450	-1.94	0.04	- 37700 - - 541.86
	BHMS429	-0.265	0.105	-1.97	0.01	- 0.470 - - 0.06
	female run date	0.0078	0.00387	1.94	0.04	0.00221 - 0.02
2006	intercept	-32700	15900	-2.4	0.04	-63800 - -1500
	BHMS29	-0.258	0.124	-1.94	0.05	-.50 - - .003
	male run date	0.0133	0.00648	2.38	0.04	0.000612 - 0.026

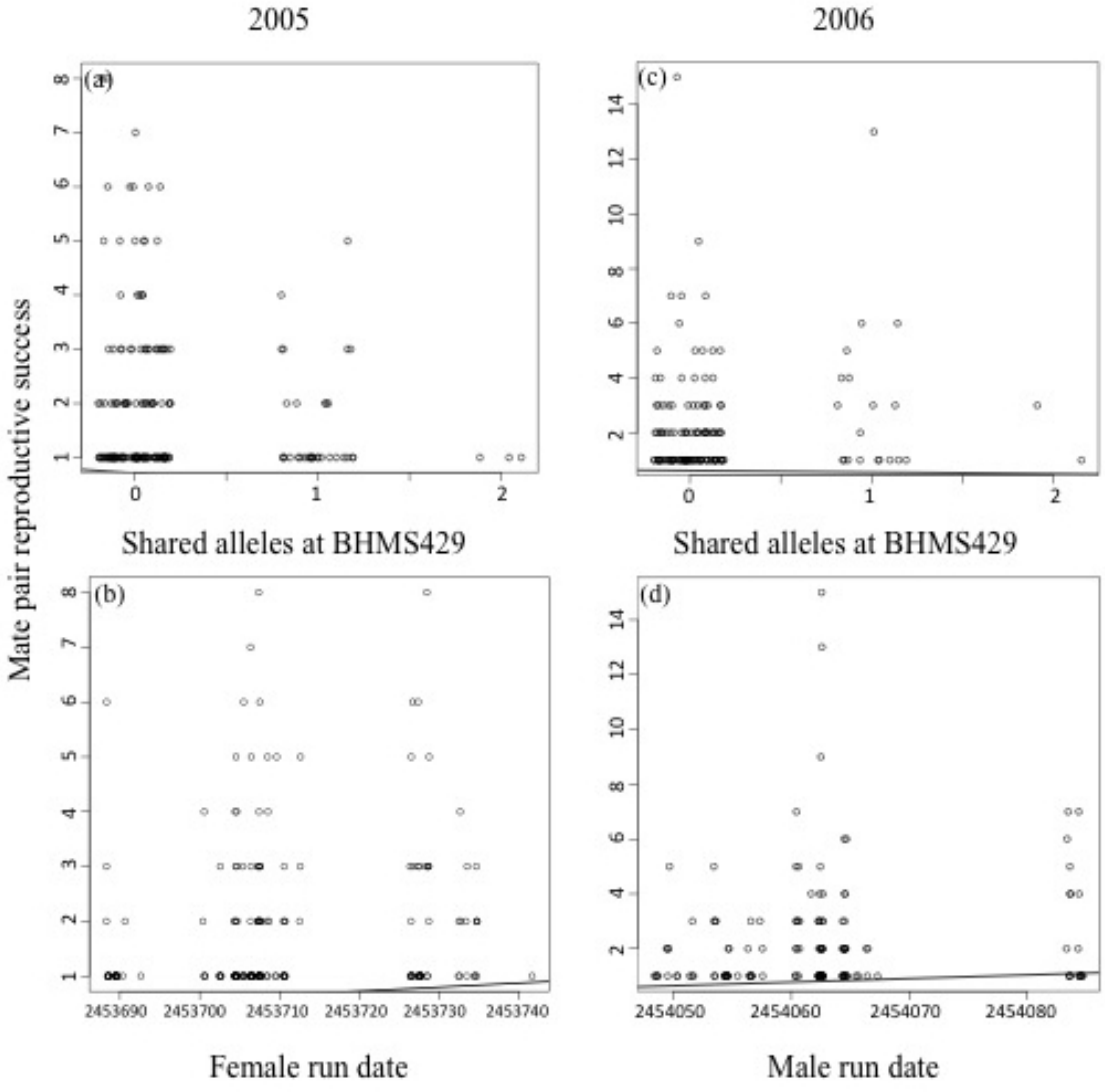

Figure 3.2: Relationship between wild x wild mate pair reproductive success and (a) *BHMS429* (b) female run date in 2005 (c) *BHMS429* and (d) male run date in 2006. Solid line illustrates the linear relationship between mate pair reproductive success and explanatory variable. Each circle represents one mate pair.

Table 3.3: Final poison log-linear regression models, determined by AIC model selection, of variables associated with 2005 and 2006 wild x hatchery mate pair reproductive success.

Year	Variable	Coefficient	Standard error	z-statistic	Two-sided P value	95% C.I.
2005	intercept	-26800	9480	-2.99	0.005	-45400 - -8220
	female fork length	0.00227	0.00112	1.83	0.04	0.0000795 - 0.00446
	male run date	0.0109	0.00387	2.99	0.005	0.00335 - 0.0185
	run date difference	-0.00973	0.00489	-1.71	0.05	-0.0193 - -0.000152
2006	intercept	-1.51	0.77	-1.80	0.05	-3.03 - 0.004
	OMM3085	0.38	0.14	4.52	0.005	0.11 - 0.65
	female fork length	0.003	0.001	2.37	0.01	0.0006 - 0.005

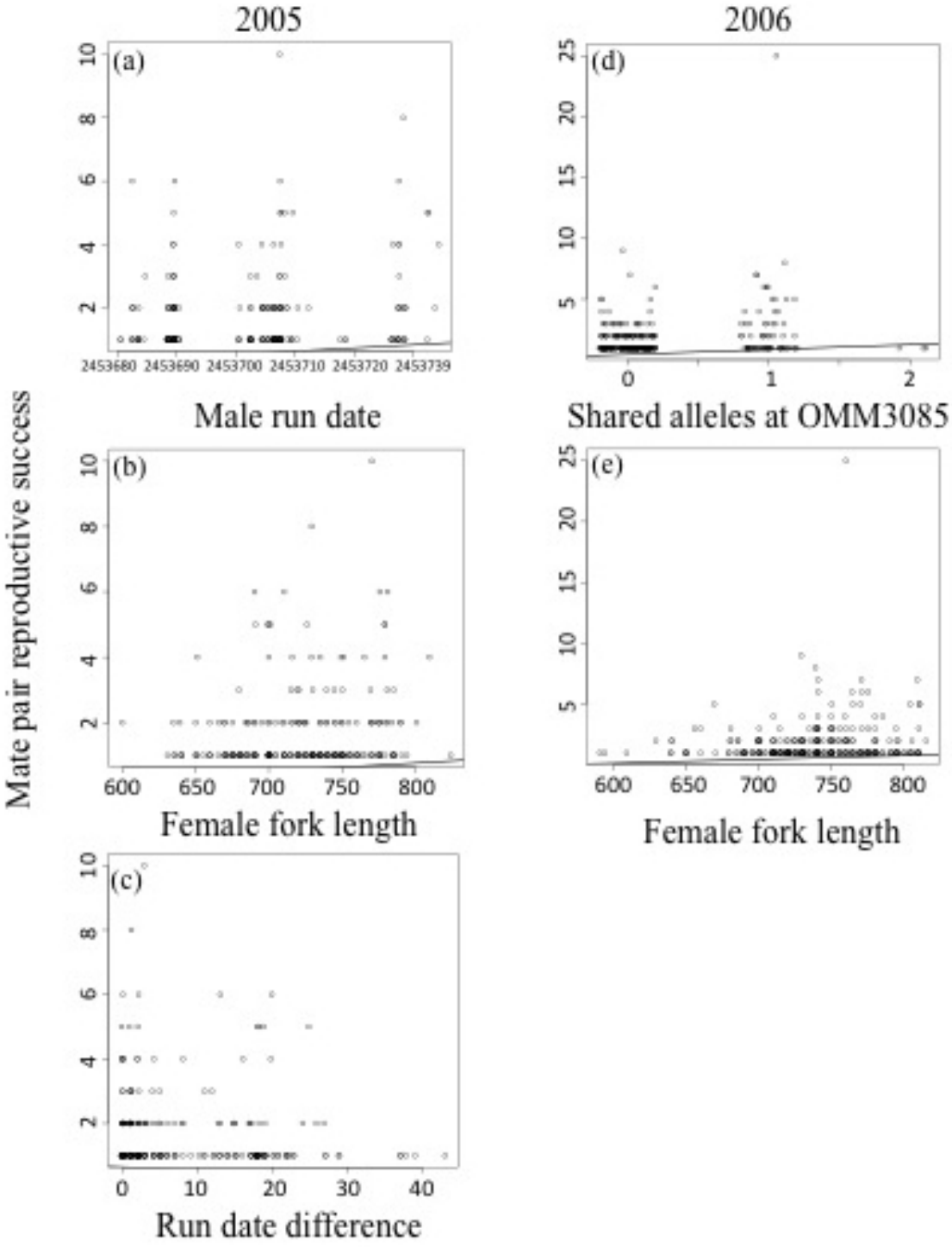

Figure 3.3: Relationship between wild x hatchery mate pair reproductive success and (a) male run date (b) female fork length (c) run date difference in 2005 (d) *OMM3085* and (d) female fork length in 2006. Solid line illustrates the linear relationship between mate pair reproductive success and explanatory variable. Each circle represents one mate pair.

< 0.40) between variables used in the final model. Female run date was correlated to male run date (Pearsons $r^2 = 0.53$), but female run date was not included in the final model. Furthermore, female run date did not have a significant relationship with mate pair RS when evaluated separately or when included in models with all parameters. When keeping the other explanatory variables in the model constant, mean mate pair RS increased by 0.03% for each 1 mm increase in female fork length ($p = 0.04$, 95% C.I. 0.00008-0.0004%). A 1-day (Julian day) increase in male run date resulted in a 0.01% increase in mean mate pair RS ($p = 0.005$, 95% C.I. = 0.003-0.02%) and a 1-day (Julian day) increase in run date difference between the male and female in each pairing resulted in a 0.01% decrease in mean mate pair RS ($p = 0.05$, 95% C.I. = 0.02-0.0002%; Table 3.3).

The model that best predicted variance in 2006 W x H mate pair reproductive success (RS) consisted of the explanatory variables female fork length and *OMM3085*, an MHC-linked marker, (Figure 3.3, Table 3.3). Therefore, our result supports the hypothesis that the number of shared alleles at immune-relevant gene-linked markers is associated with mate pair RS after accounting for additional variables that may influence mate pairing. There was a small difference between the deviance of our model and the maximum deviance of the ideal model, demonstrating no evidence of overdispersion. A goodness-of-fit chi-squared test was not significant and indicated that our model fit the data reasonably well ($p = 0.72$). There was no collinearity (Pearsons $r^2 < 0.40$) between the variables used in the final model. Female run date and male run date were correlated (Pearsons $r^2 = 0.62$) but neither variable was included in the final model and when each was eval-

uated separately for their effect on mate pair RS, both were insignificant. When keeping the other explanatory variable in the model constant, mean mate pair RS increased by 46.2% for each allele increase in the number of alleles shared at *OMM3085* between mate pairs (p = 0.005, 95% C.I. = 12-92%). A 1 mm increase in female fork length resulted in a 0.03% increase in mean mate pair RS (p = 0.01, 95% C.I. = 0.006-0.05%; Table 3.3).

3.4 Discussion

We found an association between the number of shared MHC gene-linked alleles and mate pair reproductive success (RS) for 2005 and 2006 W x W and 2006 W x H wild spawning coho salmon mate pairs. There was no association found for H x H mate pairs in either year. The fewer alleles shared at *BHMS429* between W x W mate pairs in both years was associated with increased mate pair RS, while the fewer number of alleles shared at *OMM3085* between W x H mate pairs in 2006 was associated with decreased mate pair RS.

Coho salmon mate pair RS was also influenced by other variables. Female (2005 W x W mate pairs) and male (2005 W x H mate pairs, 2006 W x W mate pairs) run date was correlated to mate pair RS, with later returning fish producing more offspring. Female fork length influenced mate pair RS for W x H mate pairs in both years, with larger females producing more offspring than smaller individuals. Lastly, the difference in run date between the male and female had a significant effect on mate pair RS for W x H 2005 mate pairs, with smaller differences between

the male and female return date resulting in higher mate pair RS.

Higher diversity of shared alleles between pairs at an MHC-linked marker has previously been reported when evaluating MHC-mediated mate choice and individual RS. Neff et al. (2008) examined genotypic correlates of RS in Chinook and found that females mated non-randomly at MHC; selecting males that are genetically dissimilar. Mate selection based on increasing offspring MHC heterozygosity was demonstrated in Atlantic salmon (*Salmo salar*) as well; although its affect on RS was not evaluated (Landry et al., 2001). Here, we report a negative association between increased mate pair RS and number of shared alleles at *BHMS429*. This is suggestive of a dissimilar mate preference, demonstrating that mate pairs more diverse at *BHMS429* had increased mate pair RS compared to those pairs more similar at *BHMS429*.

In contrast we observed a positive correlation between the number of shared alleles at *OMM3085*, another MHC-linked marker, and mate pair RS for 2006 W x H pairs. Though this trend is positive, it is likely representative of an intermediate preference since pairs sharing 1 allele (intermediate option) had the highest RS (Figure 3.3). Given that a linear analysis cannot evaluate an intermediate option, our study may have been limited by the assessment method.

Previous studies have demonstrated that intermediate MHC-heterozygosity is advantageous for offspring. This has been primarily revealed in sticklebacks (*Gasterosteus aculeatus*), when evaluating mate preference (Milinski et al., 2005; Eizaguirre et al., 2009) as well as its effect on RS (Kalbe et al., 2009). An intermediate mate preference and its advantage in terms of RS has also been shown in brown

trout (*Salmo trutta*) (Forsberg et al., 2007). Evans et al. (2012) found that while Atlantic salmon choose their mates in order to increase offspring MHC diversity, adult RS was in fact maximized between pairs exhibiting an intermediate level of MHC diversity. A recent quantitative trait loci (QTL) study showed that *OMM3085* was located within an intronic region of MHC class IA and *BHMS429* was linked to MHC class IB in rainbow trout (*Oncorhynchus mykiss*) (Rexroad et al., 2005).

Interestingly, we found no significant predictive variables for mate pair RS of H x H matings in either year. This is in direct contrast to W x W and W x H matings where, for both years, some combination of variables explained variation in mate pair RS. It is possible that our analysis did not incorporate variables that influence RS between wild spawning H x H mate pairs. For example, adipose fin length, when comparing fish of the same body size, has been previously demonstrated to play a role in mate selection (Petersson et al., 1999). It may be that wild spawning hatchery-reared fish are limited by their quality (clipped) of the adipose fin to select a mate. This may be especially true, given that additional traits unintentionally selected for in a hatchery setting (e.g.predator avoidance, Reisenbichler et al. 2004) have been demonstrated to be disadvantageous in the wild (Christie et al., 2012a). Alternatively, H x H pairs may simply lack mate choice. That is, H x H pairs mate randomly according to attributes that have been previously identified as providing offspring with a selective advantage in the wild.

The inconsistency of significant predicative variables across mating classes may have some bearing on explaining relative mate pair RS differences between mate

classes. For instance, H x H mate pair RS variance was significantly different from W x W pairs in both years (Appendix Figure D.1 and Appendix Table D.1). However, in 2005 H x H mate pair RS variance was not significantly different from W x H variance, while it was significantly different in 2006 (Appendix Figure D.1 and Appendix Table D.1). This parallels our finding that 2005 W x H mate pair RS was not explained by mate pair immune gene diversity, although it was in 2006. From strictly an immune genetic standpoint, these results suggest 2005 W x H mate pair choices were more similar to H x H mate pairs than W x W mate pair choices. Given that H x H mate pair RS was the lowest of all pair classes in both years (Appendix Figure D.1 and Appendix Table D.1) and could not be explained by any of the explanatory variables used in this study, MHC-mediated mate preferences may ultimately contribute to the root of differences in fitness between wild spawning hatchery and wild coho.

Additionally, overall fork length differences between hatchery-reared and wild fish may help to explain mate pair RS differences between the three classes (W x W, H x H, and W x H). For both years (2005 and 2006), adult male and female wild fish were significantly larger than their hatchery-reared counterparts (Appendix Table C.3). Thus, individuals involved in W x W pairs may have had an advantage in terms of mate selection. Larger coho females have an advantage in construction and competition of redds (Fleming and Gross, 1994). It has also been demonstrated that large male salmonids have increased individual RS (Williamson et al., 2010). This likely indicates the importance of male-male competition for individual RS (Quinn, 2005).

It should be noted that our model included interactions between male and female origin and respective fork lengths. None of the interactions were significant for W x H pairs. In other words, hatchery-reared females were not more likely to pair with wild or hatchery-reared males nor were wild females more likely to pair with wild or hatchery-reared males. Therefore, a component of hatchery-reared individuals pairing with each other may have just been a result of limited potential mate availability.

Peripheral variables, those that aren't directly considered in a mate's assessment, might have also affected choice. Overall fish density and differences in numbers of jacks present on the spawning grounds are two circumstantial variables that have the potential to alter or inhibit preference. Specifically, increased density, including an increase in jack presence, could affect competitive behavior and aggression (Casalini et al., 2009; Garner et al., 2010) as well as sneaker opportunities for jacks (see Section 2.4 for an explanation). Consequently, variation from year to year among these variables might explain why significant predictor variables for a mating class were not in agreement across years.

The correlation between both female (2005 W x W) and male (2005 W x H, 2006 W x W) late run time and coho salmon mate pair RS has not been previously reported when evaluating individual reproductive success in salmon. Williamson et al. (2010) identified earlier returning fish as having increased reproductive success for both males and females. Morbey et al. (2000) described the phenomenon of early male arrival in a review of four salmonid species including coho and found that early arriving males had a higher individual RS. Quinn (2005) also demon-

strated a similar advantage for males generally in terms of the operational sex ratio. However, in this study it may be the case that late arriving males saved energy by avoiding early competition with other males. Additionally, late arrival likely allows more efficient access to mating options and a guarantee that the majority of females will be present and established redds. This logic is supported by our finding that the fewer days between when the male and female returned (run date difference), the greater the mate pair RS.

The finding that female fork length was positively correlated with mate pair RS (2005 and 2006 W x H mate pairs) agrees with previous studies when examining factors that influence Pacific salmon individual RS (Quinn, 2005; Williamson et al., 2010). Large females tend to be more successful when competing for redds and they also construct deeper redds, which tend to be more resistant to disturbance (Fleming and Gross, 1994). There is evidence that body size is correlated to swimming speed (Glova and McInerney, 1977; Miller and Sadro, 2003) and larger females may also be actively finding preferable redd locations. In addition to female-female competitive advantages, increased body size, and by proxy increased swimming speed, likely aides in defending against aggressive males. Garner et al. (2010) demonstrated that aggressive behavior of Chinook salmon (*Oncorhynchus tshawytscha*) males can be an inhibitor of female mate choice. This was also demonstrated in zebrafish (Spence and Smith, 2006).

This study estimated the benefits of mate compatibility by measuring offspring survival and its relation to parental immune genetic diversity. Variables that may also influence mate pair RS were included and jointly assessed. In conclusion, we

were able to establish that MHC diversity does affect coho mate pair RS for W x W and W x H mate pairs. Later run time, small run date differences, and increased female fork length also affected W x W and W x H pair RS. No evidence for the role of any of these factors was apparent in H x H mate pair RS.

Chapter 4 – General Conclusion

Evidence for reduced reproductive success (RS) of wild spawning hatchery-reared fish (Araki et al., 2007, 2008) invites serious consideration in terms of the detrimental effects on subsequent generations of wild populations (Araki et al., 2009; Christie et al., 2012a). Mate choice was evaluated as a potential mechanism contributing to these observed RS differences using a previous pedigree of wild spawning hatchery-reared and wild origin coho salmon (*Oncorhynchus kisutch*) (Thériault et al., 2011). Two years (2005 and 2006) of three wild spawning mate pair classes were examined: wild x wild (W x W), hatchery x hatchery (H x H), and wild x hatchery (W x H). We tested for: (1) a departure from random expectations with regard to mate pair allelic diversity at immune-relevant markers, (2) a correlation between immune-relevant gene diversity and mate pair RS, and (3) distinguishable differences between mate choice strategies used by hatchery-reared and wild origin coho.

The results from this study are indicative of the complexities of mating in wild populations. For a given environmental dynamic, a number of intricate variables contribute to RS and each confer different benefits and costs (Roberts et al., 2006). Thus, our results do not always agree across Objectives, Approaches in Objective 1, and years. This makes it difficult to provide an overarching conclusion about mate choice and its effect on fitness disparities observed between hatchery-reared

and wild coho spawning in the wild. However, several findings emerge relevant to this study's goal and objectives.

The first important result is the finding that 2006 W x H mate pairs discriminated between Major Histocompatibilbity Complex (MHC) genotypes when choosing their mates (Objective 1). This was evident by a significant departure from random expectations at *BHMS429*, an MHC-linked marker, when accounting for pairs that had a RS of 0 (Approach 1) and when evaluating the type of mate preference (Approach 2). Although Approach 2 showed a similarity preference at *BHMS429*, the expected heterozygosity was greater than 0.9 for hatchery-reared and wild fish at this locus. Therefore, the generation of randomized pairs in Approach 2 would have produced mostly pairs that shared zero alleles, allowing little power to demonstrate an even greater dissimilarity. In addition, we also found a significant correlation between 2006 W x H mate pair RS and immune diversity at *OMM3085*, another MHC-linked marker (Objective 2).

In contrast, while several immune-relevant expressed sequence tags (ESTs) also displayed a non random signature in Objective 1 for 2006 W x H pairs, they were not significantly correlated to RS in Objective 2. As mentioned previously, the neutral makers (n = 10; See Section 2.2.1) used to assign parentage were also evaluated using Objective 1 methods and no significance was found. This further validates our methods and results from Objective 1.

Interestingly, comparable mate choice signals were not evident from the 2005 W x H pair results. Perhaps this is reflective of annual variation of return density to the spawning ground: 1,659 individuals were passed over Nonpareil Dam in 2005

compared to 1,442 individuals in 2006, suggesting density constraints affected mate choice decisions in 2005. Likewise, inhibition of mate choice may have been related to the presence of jacks. There was almost a twofold excess of jacks involved in 2005 successful matings compared to 2006. Jacks employ an alternate reproductive strategy, involving the avoidance of a dominance hierarchy, and instead sneak in to deposit milt during egg deposition (Gross, 1985). It is therefore likely that jacks implement an opportunistic tactic for any available female rather than mate selection. Thériault et al. (2011) reported that 2005 and 2006 hatchery-reared jacks did not exhibit the same fitness decline as their older hatchery-reared male counterparts. In other words, the fitness of hatchery-reared and wild jacks was equivalent. This finding further supports the argument that mate choice may lead to fitness disparities since jacks don't experience the same diminution.

The second major finding of this study is that the fewer alleles shared at *BHMS429*, an MHC-linked marker, between W x W (2005 and 2006) mating pairs was associated with increased mate pair RS (Objective 2). MHC-mediated mate choice has been previously demonstrated by examining MHC diversity and individual RS in Chinook (*Oncorhynchus tshawytscha*) (Neff et al., 2008) and Atlantic salmon (*Salmo salar*) (Landry et al., 2001); though its effect on RS was not evaluated in the case of Atlantic salmon. Here, we report a negative association between the number of shared alleles and mate pair RS at *BHMS429*, suggesting a dissimilar MHC-mediated mate preference.

Despite the correlation above, W x W mate pairs did not demonstrate a mate choice signature in Objective 1. This is in contrast to our findings for 2006 W x H

pairs, which provided consistent significance across both Objectives. It is possible that our significant results for W x W pairs in Objective 2 are representative of a correlation between the number of shared alleles and RS rather than mate choice. However, differences in mate pair RS observed between the three pair classes for each year may have relevance in explaining this inconsistency. W x H mate pairs had the highest RS for both years. If RS were a result of mate choice, W x H mate pairs would be most likely to exhibit a mate choice signal. So it may be that we were just unable to detect a mate choice signature in W x W mate pairs because it is a weaker signal. In fact, 2005 W x W mate pairs demonstrated a significant departure from random expectations at *BHMS429* prior to false discovery rate (FDR) corrections (Objective 1, Figure 2.5, Appendix Table A.2). Equally, 2006 W x W exhibited a significant departure from random expectations at *OMM3085* prior to false discovery rate (FDR) corrections (Objective 1, Figure 2.11, Appendix Table A.2).

Lastly, H x H mate pair RS was not correlated with any of the explanatory variables in Objective 2, whereas W x W and W x H mate pair RS was correlated to some combination of explanatory variables. It is possible that our analysis did not incorporate variables that may play a role in predicting mate pair RS for H x H mate pairs, though this is unlikely and discussed further in Section 3.4. In addition, our results are consistent with previous studies that showed an association between individual size and run date with RS (Quinn, 2005; Williamson et al., 2010). Thus, it has been well established that the explanatory variables examined here do influence RS.

The void of significant RS predictor variables for H x H pairs could also be an artifact of overall fork length differences between hatchery-reared and wild fish (male and female wild fish were significantly larger than their hatchery-reared counterparts, see Appendix Table C.3). Therefore, hatchery-reared individuals may have been limited by their size in terms of mate selection and competition. This would result in hatchery-reared individuals pairing with each other because of limited mate availability (See Section 3.4 for details). Although, the number of H x H successful matings was not disproportionately larger than W x H matings for either year, suggesting hatchery-reared fish were able to pair with wild fish. Interestingly, Christie et al. (2012b) also could not explain variation in RS when evaluating phenotypic traits (length, weight, age and run timing) in hatchery-reared wild spawning steelhead (*Oncorhynchus mykiss*). Subsequently, it may simply be that H x H pairs lack mate choice.

Previous studies have evaluated kin recognition in juvenile coho and found early rearing conditions to play an important role (Quinn and Busack, 1985; Quinn and Hara, 1986). Under the assumption that kin recognition and mate selection employ similar mechanisms (Quinn and Busack, 1985), it is possible that individuals involved in H x H pairs were affected by the high density rearing environment of a hatchery setting and therefore the ability to select a mate in the wild was altered. Given that density constraints have previously been identified as creating selective pressures in a hatchery setting (Christie et al., 2012a), this provides further evidence suggesting H x H pairs lack mate choice. An evaluation of the RS in a subsequent generation would provide additional insight (for this three-generation

pedigree we only have RS data for the F1 and F2 generation).

A recent study evaluated the fitness of wild and hatchery-reared Chinook adults reproducing in the wild and found no evidence for a decline in fitness for the supplemented hatchery group (Hess et al., 2012). One major difference between our study and Hess et al. (2012) was the ancestry of the broodstock used for the hatchery supplementation. Most hatchery programs use broodstock consisting of 70 - 80% hatchery-reared fish. Although our study created equal proportions of one to one W x W and H x H crosses in the hatchery for broodstock, the hatchery fish used for those crosses are products of an integrated hatchery management strategy that incorporated 70% hatchery-reared fish in each brood year (2002 - 2003, Figure 2.2). In contrast, Hess et al. (2012) used 100% local wild stock for all broodstock created in the hatchery each year. This likely minimized effects of adaptation to captivity as well as negative impacts on wild populations. However, proportions of returns by age class did vary between wild and hatchery-reared fish, with hatchery-reared fish returning mostly at age 3 compared to the majority of wild fish returning at age 4 (Hess et al., 2012). This suggests that hatchery rearing does have an effect on the Chinook life history (i.e. decreased age at maturity).

In this study, we were both limited and aided by only having lifetime RS (only the mate pairs that had spawning offspring are represented in these analyses) to assess mate choice. We were limited in that our evaluation of mate choice did not incorporate information on the behavioral dynamics that occurred during spawning. This represents one of the difficulties of studying mate choice *in situ* and as a result, the observed patterns reflect a combination of both pre- and postcopulatory

selection. However, evaluating mate choice by using lifetime RS as the final outcome is arguably the best measure of choice given that it is representative of the ultimate goal of spawning (to pass genetic material to ongoing generations) (Kalbe et al., 2009). In addition, length and run date for the male and female involved in each pairing were included in Objective 2 analyses to account for traits which may effect behavioral aspects of spawning (Petersson et al., 1999; Eizaguirre et al., 2009; Casalini et al., 2009; Garner et al., 2010).

As mentioned by Thériault et al. (2011), individuals straying to rivers other than the Umpqua were not accounted for when calculating RS. This could potentially effect relative RS comparisons if hatchery-reared offspring were more likely to stray than wild offspring in a wild spawning environment during 2005 and 2006. This effect is unlikely given that the hatchery-reared fish released as fry are from an integrated hatchery program and their offspring experience identical environmental conditions to their wild counterparts (Thériault et al., 2011). Along similar lines, the ancestry of the wild population was not addressed in this study. However, Thériault et al. (2011) estimated that only 15% (range 6-25%) of the wild population are likely descendants of hatchery fish released as unfed fry from previous stocking programs. Even if this were an underestimate, the observed significant difference in RS between wild and hatchery-reared fish would only be harder to detect.

This study extended the approach developed at the MHC level to test whether a non-random mate choice signature may be detected at additional immune-relevant gene-linked markers and whether that signature is associated with RS. To our

knowledge, only one other study has examined selection at non-MHC immune genes in fish populations (Jensen et al., 2007) and they did not evaluate RS. While there are inconsistencies, our study provides evidence for non-random mating in 2006 W x H mate pairs and an association between diversity at an MHC-linked marker and W x W mate pair RS. Finally, H x H mate pair RS, which was was the lowest among all pair classes in both years, was not correlated to any of the explanatory variables. Our findings suggest that MHC-mediated mate preferences may ultimately contribute to differences in fitness between wild spawning hatchery-reared and wild coho. These results are indicative of the complexities of hatchery and wild interactions and the associated fitness consequences for subsequent generations.

APPENDICES

Appendix A – Immune-relevant Marker Objective 1 Analysis

Table A.1: Calculated two-sample t-test p-values of observed and inferred pairs before and after correcting for false discovery rate (FDR) for 2005 and 2006 wild x wild, hatchery x hatchery, and wild x hatchery mate pairs at immune-relevant markers. Values prior to correction are labeled NC and those after are labeled FDR.

Marker	NC	FDR	NC	FDR	NC	FDR
2005	W x W		H x H		W x H	
OMM3026	0.450	NS	0.983	NS	0.532	NS
OMM3085	0.582	NS	0.653	NS	0.466	NS
OMM3115	0.859	NS	0.996	NS	0.108	NS
BHMS429	0.065	NS	0.086	NS	0.685	NS
SsalR010TKU	0.612	NS	0.985	NS	0.736	NS
SsalR015TKU	0.466	NS	0.119	NS	0.706	NS
SsalR013TKU	0.722	NS	0.066	NS	0.395	NS
SsalR016TKU	0.103	NS	0.258	NS	0.638	NS
2006	W x W		H x H		W x H	
OMM3026	0.898	NS	0.689	NS	0.988	NS
OMM3085	0.997	NS	0.043	NS	0.284	NS
OMM3115	0.226	NS	0.431	NS	0.399	NS
BHMS429	0.416	NS	0.729	NS	0.004	0.004*
SsalR010TKU	0.076	NS	0.696	NS	0.299	NS
SsalR015TKU	0.994	NS	0.221	NS	0.618	NS
SsalR013TKU	0.073	NS	0.107	NS	0.0001	0.0001*
SsalR016TKU	0.880	NS	0.138	NS	0.729	NS

Table A.2: Calculated mean genetic difference (MGD) p-values before and after correcting for false discovery rate (FDR) for 2005 and 2006 wild x wild, hatchery x hatchery, and wild x hatchery mate pairs at immune-relevant markers. Values prior to correction are labeled NC and those after are labeled FDR.

Marker	NC	FDR	NC	FDR	NC	FDR
2005	W x W		H x H		W x H	
OMM3026	0.999	NS	0.920	NS	0.158	NS
OMM3085	0.539	NS	0.394	NS	0.893	NS
OMM3115	0.756	NS	0.947	NS	0.028	NS
BHMS429	0.032	NS	0.211	NS	0.643	NS
SsalR010TKU	0.745	NS	0.495	NS	0.446	NS
SsalR015TKU	0.355	NS	0.495	NS	0.651	NS
SsalR013TKU	0.145	NS	0.068	NS	0.999	NS
SsalR016TKU	0.738	NS	0.092	NS	0.872	NS
2006	W x W		H x H		W x H	
OMM3026	0.346	NS	0.999	NS	0.379	NS
OMM3085	0.023	NS	0.289	NS	0.848	NS
OMM3115	0.744	NS	0.527	NS	0.941	NS
BHMS429	0.074	NS	0.808	NS	0.001	0.001*
SsalR010TKU	0.905	NS	0.747	NS	0.521	NS
SsalR015TKU	0.043	NS	0.169	NS	0.999	NS
SsalR013TKU	0.827	NS	0.234	NS	0.729	NS
SsalR016TKU	0.041	NS	0.632	NS	0.011	0.011*

Table A.3: Calculated standard deviation genetic difference (SDGD) p-values before and after correcting for false discovery rate (FDR) for 2005 and 2006 wild x wild, hatchery x hatchery, and wild x hatchery mate pairs at immune-relevant markers. Values prior to correction are labeled NC and those after are labeled FDR.

Marker	NC	FDR	NC	FDR	NC	FDR
2005	W x W		H x H		W x H	
OMM3026	0.327	NS	0.891	NS	0.048	NS
OMM3085	0.541	NS	0.535	NS	0.284	NS
OMM3115	0.431	NS	0.470	NS	0.036	NS
BHMS429	0.987	NS	0.946	NS	0.787	NS
SsalR010TKU	0.815	NS	0.308	NS	0.246	NS
SsalR015TKU	0.676	NS	0.003	0.003*	0.478	NS
SsalR013TKU	0.757	NS	0.989	NS	0.370	NS
SsalR016TKU	0.452	NS	0.934	NS	0.504	NS
2006	W x W		H x H		W x H	
OMM3026	0.546	NS	0.181	NS	0.518	NS
OMM3085	0.942	NS	0.437	NS	0.303	NS
OMM3115	0.671	NS	0.749	NS	0.557	NS
BHMS429	0.616	NS	0.569	NS	0.999	NS
SsalR010TKU	0.238	NS	0.713	NS	0.503	NS
SsalR015TKU	0.340	NS	0.873	NS	0.728	NS
SsalR013TKU	0.778	NS	0.791	NS	0.257	NS
SsalR016TKU	0.876	NS	0.465	NS	0.003	0.003*

Appendix B – Neutral Marker Objective 1 Analysis

Table B.1: Calculated two-sample t-test p-values of observed and inferred pairs before and after correcting for false discovery rate (FDR) for 2005 and 2006 wild x wild, hatchery x hatchery, and wild x hatchery mate pairs at neutral markers. Values prior to correction are labeled NC and those after are labeled FDR.

Marker	NC	FDR	NC	FDR	NC	FDR
2005	W x W		H x H		W x H	
OTS520	0.083	NS	0.434	NS	0.225	NS
OTS519	0.748	NS	0.029	NS	0.923	NS
P53	0.654	NS	0.913	NS	0.873	NS
ONE111	0.131	NS	0.452	NS	0.905	NS
OTS3	0.483	NS	0.797	NS	0.062	NS
ONEU2	0.733	NS	0.514	NS	0.742	NS
OMY1011	0.037	NS	0.667	NS	0.060	NS
ONE13	0.721	NS	0.587	NS	0.566	NS
OTS215	0.350	NS	0.150	NS	0.620	NS
2006	W x W		H x H		W x H	
OTS520	0.766	NS	0.843	NS	0.976	NS
OTS519	0.461	NS	0.346	NS	0.487	NS
P53	0.718	NS	0.056	NS	0.165	NS
ONE111	0.925	NS	0.931	NS	0.608	NS
OTS3	0.307	NS	0.806	NS	0.914	NS
ONEU2	0.382	NS	0.046	NS	0.875	NS
OMY1011	0.407	NS	0.902	NS	0.007	NS
ONE13	0.798	NS	0.385	NS	0.102	NS
OTS215	0.680	NS	0.693	NS	0.325	NS

Table B.2: Calculated mean genetic difference (MGD) p-values before and after correcting for false discovery rate (FDR) for 2005 and 2006 wild x wild, hatchery x hatchery, and wild x hatchery mate pairs at neutral markers. Values prior to correction are labeled NC and those after are labeled FDR.

Marker	NC	FDR	NC	FDR	NC	FDR
2005	W x W		H x H		W x H	
OTS520	0.075	NS	0.467	NS	0.153	NS
OTS519	0.992	NS	0.077	NS	0.344	NS
P53	0.444	NS	0.999	NS	0.746	NS
ONE111	0.334	NS	0.396	NS	0.369	NS
OTS3	0.385	NS	0.792	NS	0.041	NS
ONEU2	0.478	NS	0.451	NS	0.872	NS
OMY1011	0.092	NS	0.865	NS	0.002	NS
ONE13	0.603	NS	0.779	NS	0.165	NS
OTS215	0.556	NS	0.120	NS	0.952	NS
2006	W x W		H x H		W x H	
OTS520	0.858	NS	0.638	NS	0.931	NS
OTS519	0.703	NS	0.645	NS	0.804	NS
P53	0.906	NS	0.127	NS	0.407	NS
ONE111	0.999	NS	0.737	NS	0.411	NS
OTS3	0.967	NS	0.659	NS	0.574	NS
ONEU2	0.752	NS	0.038	NS	0.750	NS
OMY1011	0.210	NS	0.894	NS	0.054	NS
ONE13	0.826	NS	0.459	NS	0.577	NS
OTS215	0.744	NS	0.650	NS	0.740	NS

Table B.3: Calculated standard deviation genetic difference (SDGD) p-values before and after correcting for false discovery rate (FDR) for 2005 and 2006 wild x wild, hatchery x hatchery, and wild x hatchery mate pairs at neutral markers. Values prior to correction are labeled NC and those after are labeled FDR.

Marker	NC	FDR	NC	FDR	NC	FDR
2005	W x W		H x H		W x H	
OTS520	0.731	NS	0.829	NS	0.885	NS
OTS519	0.452	NS	0.124	NS	0.230	NS
P53	0.502	NS	0.562	NS	0.563	NS
ONE111	0.331	NS	0.647	NS	0.116	NS
OTS3	0.826	NS	0.485	NS	0.033	NS
ONEU2	0.635	NS	0.565	NS	0.279	NS
OMY1011	0.799	NS	0.169	NS	0.045	NS
ONE13	0.171	NS	0.119	NS	0.011	NS
OTS215	0.539	NS	0.827	NS	0.052	NS
2006	W x W		H x H		W x H	
OTS520	0.792	NS	0.219	NS	0.588	NS
OTS519	0.519	NS	0.988	NS	0.598	NS
P53	0.283	NS	0.296	NS	0.745	NS
ONE111	0.654	NS	0.895	NS	0.959	NS
OTS3	0.621	NS	0.712	NS	0.227	NS
ONEU2	0.556	NS	0.867	NS	0.157	NS
OMY1011	0.924	NS	0.719	NS	0.550	NS
ONE13	0.858	NS	0.227	NS	0.859	NS
OTS215	0.276	NS	0.521	NS	0.723	NS

Appendix C – Hatchery and Wild Population Comparisons

Table C.1: Observed (Ho) and expected (He) heterozygosity parameters for both immune-relevant gene-linked and neutral markers per locus and per type of fish (hatchery-reared or wild) for each year. Calculated using Genetix (Belkhir et al. 2004).

	05 wild		05 hatchery		06 wild		06 hatchery	
Locus	He	Ho	He	Ho	He	Ho	He	Ho
OMM3026	0.593	0.606	0.557	0.553	0.537	0.507	0.525	0.553
OMM3085	0.874	0.793	0.879	0.819	0.897	0.821	0.909	0.840
OMM3115	0.945	0.828	0.945	0.832	0.951	0.783	0.949	0.748
BHMS429	0.947	0.836	0.951	0.839	0.951	0.759	0.953	0.716
SsalR010TKU	0.726	0.721	0.761	0.776	0.776	0.747	0.786	0.727
SsalR013TKU	0.805	0.236	0.846	0.407	0.797	0.347	0.842	0.348
SsalR015TKU	0.699	0.698	0.681	0.702	0.735	0.729	0.723	0.702
SsalR016TKU	0.863	0.459	0.865	0.407	0.858	0.294	0.875	0.365
OTS520	0.924	0.951	0.927	0.938	0.922	0.931	0.931	0.939
OTS519	0.714	0.747	0.762	0.752	0.767	0.765	0.773	0.755
P53	0.869	0.848	0.842	0.819	0.857	0.867	0.852	0.865
ONE111	0.645	0.701	0.668	0.655	0.629	0.599	0.687	0.759
OTS3	0.825	0.764	0.813	0.792	0.821	0.783	0.828	0.773
OCL8	0.888	0.899	0.907	0.904	0.888	0.883	0.893	0.887
ONEU2	0.943	0.914	0.957	0.907	0.936	0.736	0.942	0.699
OMY1011	0.873	0.888	0.863	0.848	0.847	0.831	0.844	0.851
ONE13	0.919	0.891	0.894	0.904	0.878	0.859	0.870	0.784
OTS215	0.693	0.644	0.689	0.658	0.701	0.624	0.720	0.692

Table C.2: Fst for immune-relevant gene-linked markers observed between 2005 and 2006 hatchery-reared and wild origin fish. Calculated using Genetix (Belkhir et al. 2004).

	05 wild fish	06 hatchery fish	06 wild fish
05 hatchery fish	0.006	0.024	0.019
05 wild fish		0.024	0.016
06 hatchery fish			0.004

Table C.3: Fork length comparisons between 2005 and 2006 hatchery-reared and wild origin fish by sex. In both years and for each sex wild fish are larger. Calculated using two-tailed two-sample t-tests in R v. 2.13.2 (R Development Core Team, 2011.

Sex	Year	p-value
Male	2005	0.001
	2006	0.008
Female	2005	0.0003
	2006	0.05

Appendix D – Mate Pair Reproductive Success Differences

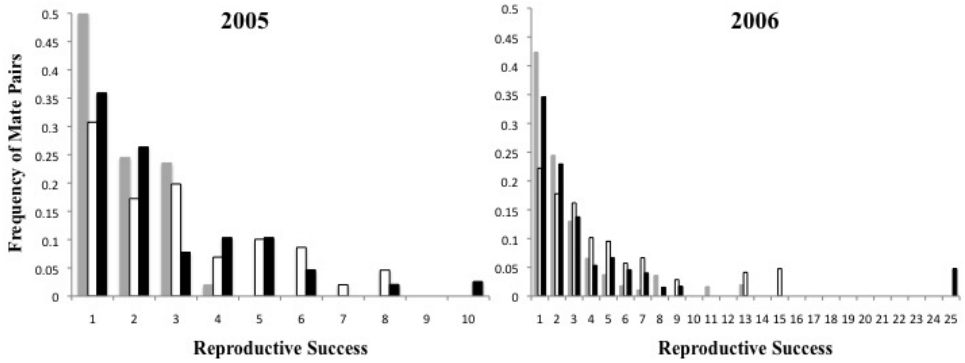

Figure D.1: Histograms of mate pair reproductive success for 2005 and 2006 wild coho returns. The frequency is standardized by the total number of mate pairs in each pair class. Gray columns = hatchery x hatchery mate pairs, white columns = wild x wild mate pairs, and black columns are wild x hatchery mate pairs.

Table D.1: Levene's test differences in mate pair reproductive success variance between wild x wild, hatchery x hatchery, and wild x hatchery mate pairs for 2005 and 2006.

	2005		2006	
	W x W	W x H	W x W	W x H
H x H	0.00001	0.08	0.002	0.03
W x W		0.03		0.48

Appendix E – Mate Pairs that Involve Jacks

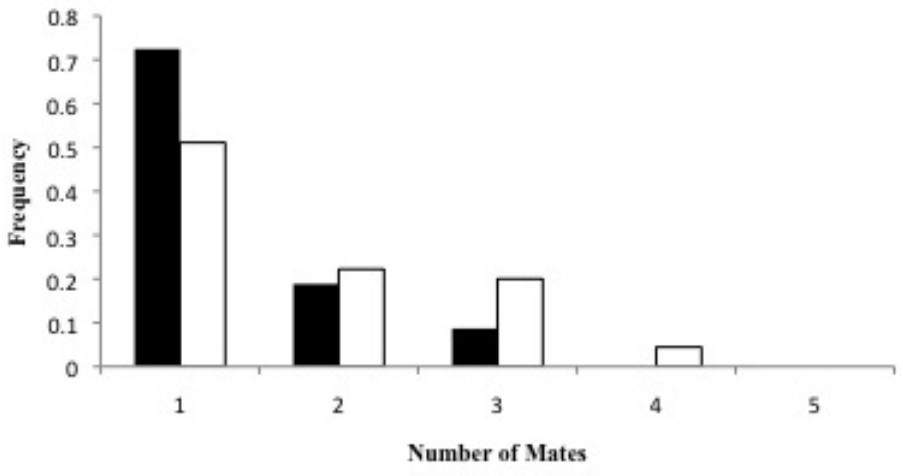

Figure E.1: Distributions of the number of mates for 2005 (black columns) and 2006 (white columns) jacks, they are significantly different (Levenes Test p = 0.006). The frequency is standardized by the total number of jacks in each year.

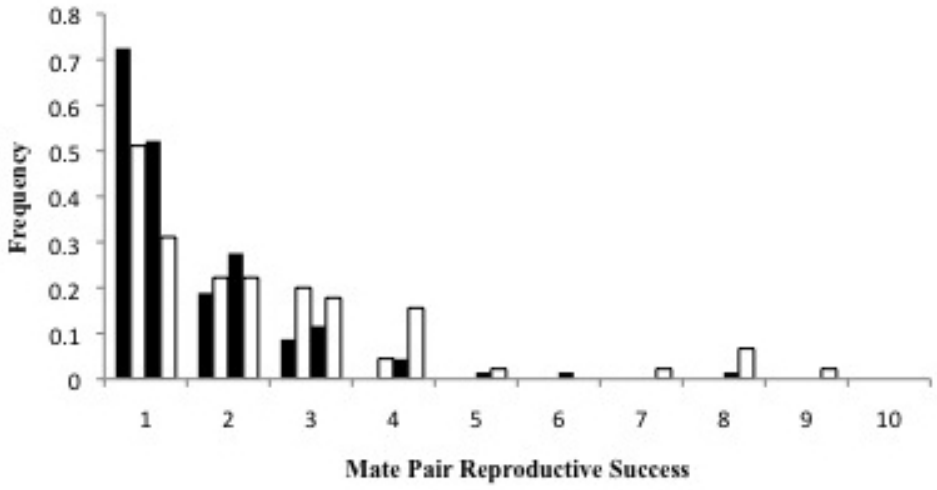

Figure E.2: Histograms of mate pair reproductive success for pairs that involved a jack for 2005 (black columns) and 2006 (white columns), they are significantly different (Levenes Test p = 0.02). The frequency is standardized by the total number of pairs in each year.

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